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SOIL SCIENCE

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NOTE ON THE RELATION BETWEEN LIME CONTENT AND pH VALUES OF SOILS

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During the autumn of 1931 a large number of samples were taken for the purpose of making a soil survey, and determinations were made of lime content and pH, both in aqueous and in normal potassium chloride extracts of soils. The latter were determined colorimetrically according to the $BaSO_4$ method (5) using the complex indicators I and II as supplied by H. Jurany.[1]

The soils, which varied widely in their origin and composition, ranged from those with a high content of organic matter to those with none. Such widely different soil types as podzols and alkaline soils were also included so that as

TABLE 1

pH differences (H_2O and KCl) in extracts of soils in relation to the lime content

pH DIFFERENCE	NUMBER OF SAMPLES CONTAINING CALCIUM CARBONATE			TOTAL NUMBER OF SAMPLES
	0 per cent	0–0.6 per cent	>0.6 per cent	
<0.3	58	1	4	63
0.4–1.0	188	30	48	266
>1.0	22	9	34	65
Total.....	268	40	86	394

wide a range as possible was obtained for the purpose of testing both the reliability of the pH methods as devised by the author and the variations in pH obtained by using potassium chloride for extraction.

As a result of applying the aforementioned methods of determining pH, it has been found that an interesting correlation exists between the divergent results obtained with the aqueous and potassium chloride extracts and the lime content of the soil. Because of the drift that occurs with the quinhydrone electrode, the regularity is not observed when the latter method is used (2, 3, 4).

The close relation between the lime content and pH difference may be seen from the data of table 1. The data prove that the great pH divergences oc-

[1] H. Jurany, Budapest IV, Váci utca 40.

cur with the alkaline, i.e. saturated, soils; minor divergences, with the limeless, i.e. unsaturated, soils.[2]

The surprising relation between the pH and pH difference may be seen in table 2 and figure 1. Neutral soils show a minimum divergence, and the

TABLE 2

pH of the soils

	NUMBER OF SAMPLES					
	7	31	100	82	34	27
pH	5.0–5.4	5.5–5.9	6.0–6.4	6.5–6.9	7.0–7.4	7.5–7.9
Average pH difference with KCl and HP	0.63	0.75	0.60	0.44	0.49	0.71

	NUMBER OF SAMPLES				
	39	34	11	26	3
pH	8.0–8.4	8.5–8.9	9.0–9.4	9.5–9.9	10.0–10.4
Average pH difference with KCl and HP	0.78	0.95	1.12	1.28	1.43

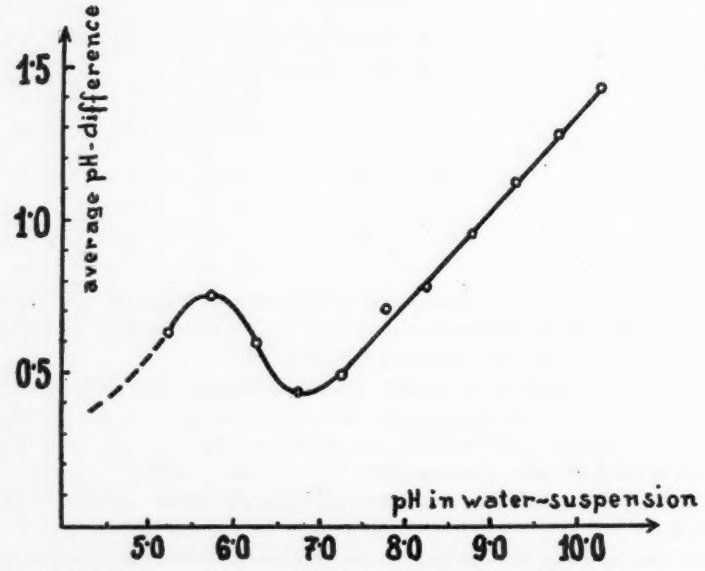

FIG. 1. RELATION BETWEEN pH VALUES AND AVERAGE pH DIFFERENCES

[2] There has been found a slight (<0.3 pH) difference with 268 limeless samples in 58 cases (21.6 per cent) and with 86 strongly calcareous soils only in 4 cases (4.7 per

difference is greatest with strongly alkaline soils. A point of inflection is obtained at pH 5.5 — 6.0 but the divergence diminishes at acidities greater than pH 5.5. Deines and Kleinschmit (1), however, found that at stronger acidities the average divergence is still less. Although no theoretical explanation can be given for the results, the regularity of the curve points to definite correlation between the aqueous pH and the pH as obtained in potassium chloride extracts. The data certainly refute the assumptions hitherto existing that small pH divergences occur with the saturated soils and great pH divergences with the unsaturated.

SUMMARY

A comprehensive examination of the pH results obtained in aqueous and 1*N* KCl extracts of 394 soil samples showed that:

Acid soils seldom show the large differences obtained with the highly alkaline soils.

A certain degree of regularity is observed between the average pH difference and the aqueous pH.

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cent). Great divergence (>1.0 pH) has been found in 22 cases out of 268 samples of limeless soils (8.2 per cent), and in 34 cases out of 86 samples of strongly calcareous soils (39.5 per cent); that is, five times as frequently.

A NOTE ON THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF SOIL COLLOIDS AND TWO OF THEIR PROPERTIES[1]

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Recent studies on the factors influencing the ratio of the moisture equivalents of soils to their permanent wilting percentages (4) have focused attention upon the chemical composition of the colloidal fractions of those soils.

Although Briggs and Shantz suggested a value of 1.84 for this supposed constant, more recent work by Veihmeyer and Hendrickson (9) demonstrates that no single value is acceptable for all soils. These workers report values ranging from 1.73 to 3.82. Local work (10) indicates that for Hawaiian soils a ratio of 1.30 is fairly accurate for most soils, although, as has been pointed out (19), each soil must be independently studied. It is significant that most of the soils reported by Briggs and Shantz[2] were obtained from the Great Plains Area; the highest ratio of Veihmeyer and Hendrickson (9) was from an Ohio soil; the low values obtained from local work are from soils which tend to be lateritic in view of the tropical conditions under which they were formed. Since the work of Gile (5), Anderson and Buyers (1), Robinson and Holmes (8), and others indicates that the chemical composition of the colloidal fraction varies with the conditions of weathering, a study of the physical significance of these variations was undertaken.

MOISTURE EQUIVALENT OF COLLOIDS AND THEIR CHEMICAL COMPOSITION

In 1930 Bouyoucos (2) suggested a direct proportionality between the colloidal contents of soils, as measured by the hydrometer, and their moisture equivalents. One is left with the impression that the water holding capacity of a soil, as measured by its moisture equivalent, depends upon the amount of colloidal material present, and that soil particles larger than the arbitrary limit for "colloidal material" act as simple adulterants and supply no water retaining power of their own. Apparently Briggs and McLane (3) had reached the same conclusion. On the other hand, Middleton (7) reported that although there was a general increase in the value of the moisture equivalent as the percentage of colloidal material increased, no precise relationship existed. It is significant that Middleton's results were based on 172 soils from various parts of the United States.

[1] Published with the permission of the director, Hawaii Agricultural Experiment Station.

[2] Personal correspondence with H. L. Shantz in 1932.

Bouyoucos' (2) results are plotted in figure 1. Here there is a significant straight line relationship for 11 of his soils. Moreover, the straight line when extended passes through the origin. But three of his points, which he dismisses as being "organic," are in themselves essentially collinear.

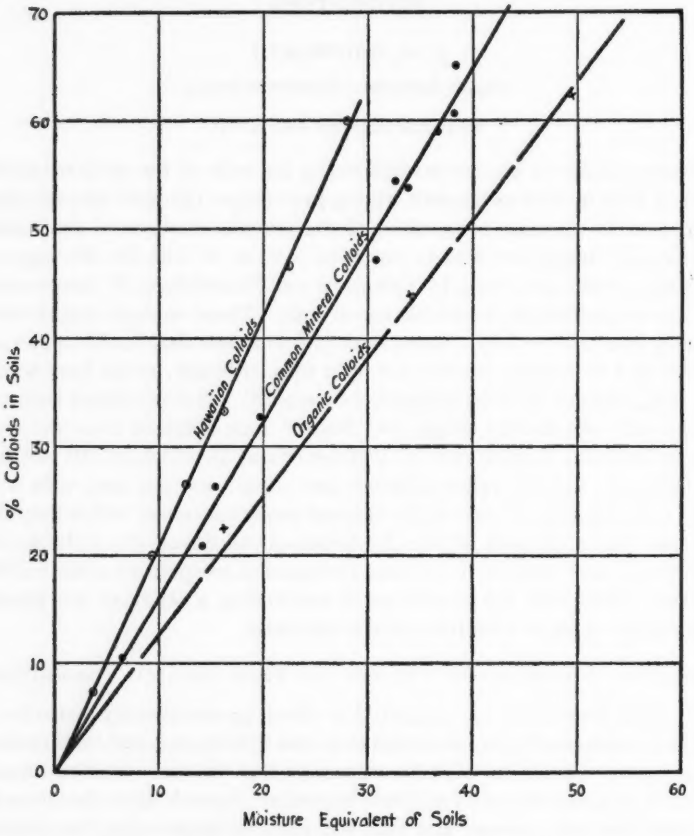

FIG. 1. RELATION BETWEEN THE MOISTURE EQUIVALENTS OF SOILS AND THE PERCENTAGES OF COLLOIDAL MATERIALS

Solid dots from general run of Bouyoucos' (2) results. Crosses from soils classified by Bouyoucos as "organic." Circles, "soils" formed by adding given amounts of extracted colloids from Hawaiian soil to silica sand.

Hawaiian soils do not respond readily to the hydrometer method because of the difficulty of obtaining comparable dispersion. Consequently "synthetic" Hawaiian soils were prepared by adding known percentages of extracted colloids to a base of inert silica sand of such grading that its effect

on the moisture equivalent might be ignored. Again the points are collinear, but although the line passes through the origin its slope is quite different from that of either of the other two.

If confidence can be placed in the few observations available for use in figure 1, one might conclude that the Bouyoucos proportionality between the colloidal contents and the moisture equivalents of soils would hold only if the colloidal materials involved in all cases were similar with respect to their water holding capacities. Furthermore, from the slopes of the three lines in figure 1 one might assume that unit mass of organic colloid held a relatively large amount of moisture at the moisture equivalent; unit mass of colloid from the Bouyoucos soils, less; and unit mass of colloid from Hawaiian soils, least. Since Hawaiian colloids are relatively lean in silica and rich in iron and alumina, it might be reasoned further that colloidal silica and organic matter had relatively high water retaining capacities but that colloidal iron and alumina did not.

Opportunity for testing this assumption is available in the recent work of Anderson and Buyers (1), who report the chemical nature of the colloidal material extracted from soils from six different series together with some of the physical properties of the same separates. The series are well selected, the Amarillo series being typical of the Great Plains, the Superior and Beckett series being classified as podzols, and the Davidson and Nipe series and bauxite representing semi-tropical conditions. In view of the samples obtained from various points in the profile, 24 samples are reported in detail.

Gile (5) and others have reported significant lineal correlations between the physical properties of soil colloids and the SiO_2/Al_2O_3 and $SiO_2/Al_2O_3 + Fe_2O_3$ ratios of those colloids. In the present case the correlation between the moisture equivalents of the 22 colloids available and the corresponding SiO_2/Al_2O_3 ratio is $+ 0.732 \pm 0.070$. Significant as this coefficient may be, it may be improved by the use of the more conventional molar ratio of silica to iron plus alumina. When these ratios are compared with the observed moisture equivalents, a correlation coefficient of $+ 0.751 \pm 0.062$ is obtained.

This increase in the correlation coefficient naturally suggests that colloidal iron plays a part in fixing the moisture equivalent of the colloidal material. However, the use of $Al_2O_3 + Fe_2O_3$ in the denominator assumes that mole for mole iron and alumina carry the same moisture content when at the moisture equivalent. This may be far from the truth. Moreover, the organic matter, which seems to be high in water holding capacity, is still ignored.

A modification of the procedure permits the inclusion of all four variables which seem to dominate the water holding capacity at the moisture equivalent. In this procedure colloidal silica, iron, and alumina are assumed to exist as uncombined materials in the colloidal complex. Each is endowed with an unknown capacity for holding water at the moisture equivalent. The sum of each of these unknown capacities times the percentage of the proper

constituent of the colloid should give a measure of the moisture equivalents. Or

$$\text{M.E.} = x\ SiO_2 + y\ Al_2O_3 + z\ Fe_2O_3 + w\ \text{Organic}$$

where x, y, z, and w are the water holding capacities of SiO_2, Al_2O_3, Fe_2O_3, and organic matter respectively, and SiO_2, Al_2O_3, etc. represent the percentage of these materials in the colloid as disclosed by the analysis.

In the material at hand (1) we have the opportunity of forming 22 equations of this form. As has been indicated, the soils involved cover an extended range. The most plausible values for each of the unknowns may be determined by the method of normal equations. When this is done the following values are obtained:

	Per cent
x = m.e. of colloidal SiO_2 in soil	140
y = m.e. of colloidal Al_2O_3 in soil	31
z = m.e. of colloidal Fe_2O_3 in soil	61
w = m.e. of colloidal organic matter in soil	116

If these results are acceptable it is evident that colloidal silica and organic matter are particularly important in determining the moisture equivalent of soil colloids, as has been pointed out in the discussion of Bouyoucos' results.

When these values are substituted in the original 22 analyses, we may compute the value of the moisture equivalent with a precision which, when compared with the observed values, measures the validity of the assumptions. When such computed values are compared with the observed values, a correlation coefficient of $+0.859 \pm 0.037$ is obtained. Such a high value, in view of the fact that the moisture equivalent of such finely divided material can be determined only with difficulty, may be considered as decidedly significant, particularly since all oxides of minor occurrence have been ignored.

EXCHANGEABLE BASE CAPACITY AND COLLOIDAL COMPOSITION

The data by Anderson and Buyers (1) permits the drawing of similar correlations between the exchangeable base capacity for the several colloids listed and their chemical composition in accordance with the method used in the foregoing. Figures for exchangeable base capacity are given in milliequivalents per gram of colloid by $BaCl_2$.

In comparing the usual $SiO_2/Al_2O_3 + Fe_2O_3$ ratio with exchange capacity in 23 cases, a correlation coefficient of $+0.855 \pm 0.038$ is obtained. If the simpler ratio of SiO_2/Al_2O_3 is used, the correlation coefficient increases to $+0.876 \pm 0.033$. Computing the ratio once more as $SiO_2 + Fe_2O_3/Al_2O_3$ reduces the correlation coefficient to $+0.574 \pm 0.094$. If significance can be claimed for these differences in the correlation coefficients, there seems to be some evidence that SiO_2 and Al_2O_3 differ widely in their base exchange characteristics, whereas Fe_2O_3 is relatively inert.

Resorting to the procedure used in the interpretation of the moisture equiva-

lent and assuming again that the materials exist in simple uncombined forms, we may write,

Base capacity = x per cent SiO_2 + y per cent Al_2O_3 + z per cent Fe_2O_3 + w per cent organic

Twenty-three cases reported provide an equal number of equations in this form. The procedure of normal equations again produces the most plausible value. When this is done

$x = +0.01570$ = Exchangeable base per gram SiO_2
$y = -0.01044$ = Exchangeable base per gram Al_2O_3
$z = +0.00033$ = Exchangeable base per gram Fe_2O_3
$w = +0.00911$ = Exchangeable base per gram organic matter

When these values are substituted in the 23 original equations and the computed base capacity is compared with the observed value, a correlation coefficient of $+0.919 \pm 0.025$ is obtained.

Significant as this relation seems to be, it is based upon values which are rather unexpected. The positive values for x, w, and z indicate that SiO_2, organic matter, and Fe_2O_3 possess base exchange capacities in the order named. The negative value for Al_2O_3 suggests that this material not only fails to possess base exchange capacity but actually tends to depress that characteristic in material adjacent to it. As an example, in the surface foot of the Nipe series a colloid carrying 10.2 per cent SiO_2 and 62.5 per cent Fe_2O_3 gave an observed base exchange capacity of only 0.031 m.e. Here the exchange capacity of 0.180, upon the basis of the values already given, is reduced to an observed value of 0.031, possibly because of the 15.8 per cent Al_2O_3 present.

If the negative sign for the Al_2O_3 may be accepted, it would be reasonable to suppose that this material is particularly or exclusively active in anion absorption. In fact, Matson (6) attacking the problem from an entirely different angle, reports that "with an increase in the silica content of the colloid we find an increase in the cation absorption and a decrease in anion absorption." And again, "As the SiO_2/R_2O_3 ratio increases, the colloids assume a more pronounced electronegative character. . . . ."

SUMMARY

This paper is a statistical review of recent literature dealing with the relation between the chemical nature of soil colloids and their physical properties.

From the evidence available, there seems to be no reason for belief that SiO_2, Al_2O_3, and Fe_2O_3 exist in soil colloids in any form other than the simple oxides or hydrated oxides. Moreover, in so far as such statistical studies may be accepted, it would appear that each of these materials, as well as any organic matter which may be in colloidal form, possesses definite water holding characteristics at the moisture equivalent. When the chemical composition of a colloid is known, its moisture equivalent should be easily determined.

The base exchange capacity of colloids seems to be a function of their chemical composition as well. SiO_2, Fe_2O_3, and organic matter appear to absorb

these cations, whereas alumina seems to repel cations and possibly absorbs anions.

If it be true that the moisture equivalent of a soil is determined by the abundance and chemical nature of the colloidal material in it, it would seem reasonable to suppose that the moisture in a soil at the permanent wilting percentage is a function of the same variables. Consequently, a constant ratio between moisture equivalents and wilting percentages for all soils could exist only if the moisture held by each colloidal component were reduced by the same percentage during the growth of plants, prior to evidence of wilt.

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THE TOXIC INFLUENCE OF FLUORINE IN PHOSPHATIC FERTILIZERS ON THE GERMINATION OF CORN[1]

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Marked reduction in the percentage of germination of seeds of cultivated plants has often resulted from the application of fertilizers in contact with or near the seed. The causes and remedy for this injury have been the subject of numerous investigations. The Committee on Fertilizer Placement of the National Fertilizer Association (7) and coöperating agricultural experiment stations have accomplished much to remedy this objectionable effect by determining safe and effective methods of fertilizer application. Some excellent work has also been done to determine some of the causes of this fertilizer injury. It was believed, however, that there was need for further knowledge concerning this matter; therefore, the investigation described in this paper was undertaken.

HISTORICAL

The literature is replete with observations of retardation and inhibition of germination and growth caused by fertilizers and other chemical compounds. As early as 1733 Jethro Tull (14) noted that "too much nitre corrodes a plant." That was approximately one hundred years before the first shipment of Chilean nitrate from Iquique. Late in the nineteenth century an active interest was taken in the problem by French and German investigators. These early investigations were concerned chiefly with laboratory tests using solutions of single salts.

Claudel and Crochetelle (3) investigated the effect of KCl, K_2SO_4, $(NH_4)_2SO_4$, $NaNO_3$, ammoniated superphosphate, and basic slag on the germination of seeds. All of these materials, at the concentrations used in their experiment, except basic slag (which is relatively insoluble) retarded germination.

Vincent (15), working with wheat seeds and several soluble fertilizer salts, concluded that the ability to germinate was decreased with increase in concentration of the salt.

Much later, in 1921, Rudolfs (9) showed that the retarding action, with some exceptions, could be attributed to an interference with absorption of water by the seed as a result of the high osmotic concentration of the salt solutions.

Numerous other workers employing solutions and pot and field tests have supported the

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[1] Summary of a thesis submitted to the faculty of Michigan State College in partial fulfillment of the requirements for the degree of doctor of philosophy. Published as journal article 180 (n.s.) of the Michigan Agricultural Experiment Station.

[2] The writer wishes to express his appreciation to Dr. C. H. Spurway and Dr. C. E. Millar for helpful suggestions and criticisms during the progress of the work and the preparation of the manuscript. Thanks are due to other members of the soils department and the Experiment Station for their kindly interest and advice.

findings of these men. Little doubt remains that high osmotic concentrations have a retarding effect on germination.

Injury to germination can be caused, however, not only by high osmotic concentration, but also by toxic substances.

Sigmund (12), working with peas, corn, and rape seeds, found that a 0.5 per cent solution of KF entirely prevented germination.

Bokorny (2) found that a 0.1 per cent solution of NaF was very injurious to cress seedlings and that a 0.1 per cent solution of HF completely prevented the germination of cress, barley, peas, flax, and bean seeds.

It is well known that phosphate rock contains a considerable quantity of fluorine. The analyses by Jacobs and Reynolds (5) and also of Marshal and co-workers (6) show that the fluorine content of rock phosphate ranges between 3 and 4 per cent for most of the samples analyzed. Moreover, the first mentioned investigators showed that most of this fluorine is carried over into the product during the manufacture of superphosphates, and the latter workers found that some fluorine is present in the phosphoric acid used for making treble superphosphate.

Allison (1), growing corn in tumblers, found that heavy applications of 18 per cent superphosphate prevented germination.

Rost (8), in pot and field work, found that both 16 and 46 per cent superphosphates were injurious to corn at heavy rates of application. The 46 per cent superphosphate was more toxic in equivalent amounts than the 16 per cent superphosphate. Contact with the soil for 1 month before planting largely overcame the toxicity of both fertilizers.

EXPERIMENTAL

Toxicity of superphosphates to germination of corn

To investigate the toxicity of various fertilizer materials, a method was used similar to that used by bacteriologists in testing the efficiency of disinfectants. The organisms (corn seeds in this case) were placed for a definite number of hours in the material investigated and then placed in a suitable medium for growth. The effect of the treatment on viability was then compared with a control run parallel with the treated organisms.

Pastes were made by moistening fertilizers with distilled water. Each paste was placed in a 2-pound glass butter dish having a loose glass cover. One hundred seeds of corn were embedded in each of the pastes for the time designated. Corn seeds of uniform size and high viability were used. Any seeds that showed indication of mechanical injury or poor quality were discarded. After remaining in the paste for a definite number of hours, the seeds were removed, well washed with distilled water, weighed, and placed in "rag dolls" to germinate. For the fluid treatments the same procedure was followed except that 500-cc. Ehrlenmeyer flasks, stoppered with absorbent cotton, were used as containers.

The "rag doll" consisted of a strip of cheesecloth 8 inches wide and 30 inches long. The hundred seeds were spread uniformly on each strip and then rolled up with the cloth, a glass rod being used as an axis to give stiffness to the roll. Each "rag doll" was placed in a 5-pint jar containing 1 inch of distilled water so that the wick action of the cheesecloth moistened the seeds. The jars were then connected by means of rubber tubing and aerated with moist air four times

daily. Germinated seeds were removed daily, and the number that germinated was recorded. As the reaction of the medium for plant growth may influence germination, the pH values of the pastes and solutions were measured by means of the quinhydrone electrode. The results of the experiment are given in table 1.

These results show that commercial ammonium sulfate and potassium chloride were injurious to germination. The toxic effect produced by the two superphosphates was especially severe. Immersion in these two materials for 3 hours was sufficient to reduce germination 75 per cent, and immersion for 12 hours killed practically all of the seeds. Similar toxicity was not shown by monocalcium phosphate, phosphoric acid, or sulfuric acid in the concentrations

TABLE 1

Per cent germination of corn after immersion in fertilizer materials

TREATMENT	SUBSTANCE	CONCENTRATION OF MEDIUM	pH OF MEDIUM	NUMBER OF HOURS IMMERSED					
				1	3	6	12	24	96
				Per Cent Germination					
1	H_2O (distilled)	.....	....	..	..	..	..	98.5*	..
2	$(NH_4)_2SO_4$ (com'l)	Paste	4.23	..	..	..	93	85	88
3	KCl (com'l)	Paste	6.90	..	..	..	84	77	65
4	Superphosphate 20 per cent P_2O_5	Paste	2.48	87	27	7	1	0	0
5	Superphosphate B 44 per cent P_2O_5	Paste	2.41	86	24	9	0	0	0
6	$Ca(H_2PO_4)_2$ C. P.	Paste	1.56	..	..	..	..	91	..
7	H_3PO_4	0.1 *M*	1.61	..	..	..	..	99	..
8	Rock phosphate and H_2O	5 gm./100 cc.	7.16	..	..	..	..	97	..
9	H_2SO_4	0.143 *N*	1.20	..	..	..	..	95	..
10	Rock phosphate and 0.143 *N* H_2SO_4	5 gm./100 cc.	1.86	..	..	..	..	0	..

* Average of 10 determinations of 100 seeds each. Standard deviation = 1.12 per cent.

used. Neither was it shown by rock phosphate in water. When rock phosphate and 0.143 *N* sulfuric acid were mixed, however, a product was obtained that was highly toxic to germination.

Since retardation of germination due to high osmotic concentration of the medium is related to the water absorption of the seed, the weight of seeds before and after immersion in the fertilizer pastes was recorded. The per cent increase in weight of the seeds is shown in figure 1. It is at once apparent that the phosphates interfered with water absorption much less than did the more soluble ammonium sulfate and potassium chloride which, in turn, were less toxic than the phosphates. The interference with water absorption, then, does not afford an explanation of this injury.

The high acidity of the phosphates evidently did not account directly for their toxicity under the conditions of the experiment, because the more acid phosphoric and sulfuric acids did not produce a similar effect. Therefore, an explanation of the injury must be sought elsewhere.

Qualitative tests showed that concentrated sulfuric acid formed hydrofluoric acid from rock phosphate. When the mixture was heated a heavy

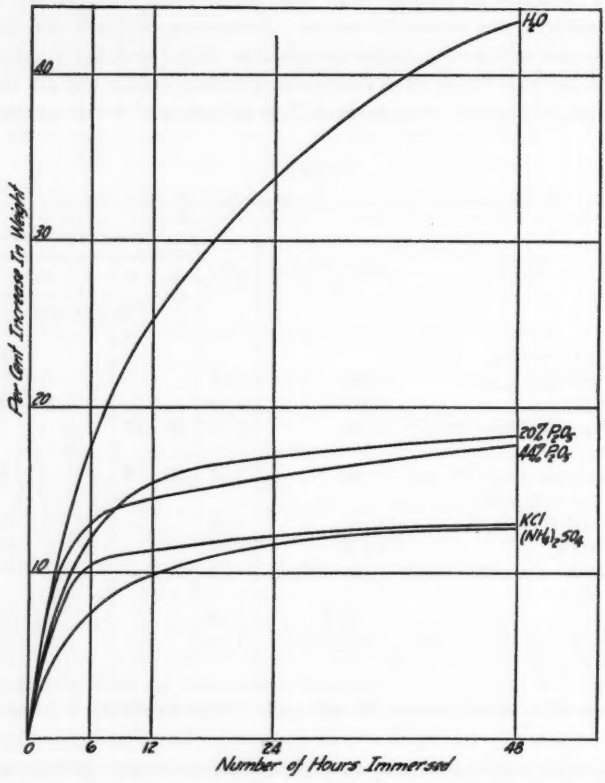

FIG. 1. PERCENTAGE INCREASE IN WEIGHT OF CORN SEEDS PLANTED IN MOIST FERTILIZER

etching was produced on a glass plate. Therefore, it seemed desirable to investigate fluorine as a possible source of injury to germination.

Toxicity of some fluorine compounds to germination of corn

Solutions of hydrogen fluoride and sodium fluoride were made containing known concentrations of fluorine. The strengths of these solutions were checked by titration with 0.1 *N* thorium nitrate using alizarin as an indicator, according to the method of Willard and Winters. The pH values of the solutions were determined by means of the quinhydrone electrode.

Four hundred cubic centimeters of each solution were placed in separate 5-pint jars. In each of these were placed two "rag dolls" each containing 100 seeds of corn. One of these "rag dolls" contained Polar Dent corn (the same variety used in the previous experiment), the other contained Pickett's Yellow Dent corn. In addition to the jars containing the fluoride solutions four more were added to the series. One of these contained 400 cc. of 0.143 *N* H_2SO_4; the second, an equal volume of 0.072 *N* H_2SO_4; the third, 400 cc. of 0.072 *N* H_2SO_4 plus 1 gm. of CaF_2 per 100 cc. of solution; and in the fourth jar solid CaF_2 was sprinkled directly on the seeds in the "rag doll" and 400 cc. of water was added. The jars were connected in series by tubing, aerated, and otherwise treated as in the first experiment.

TABLE 2

Influence of some fluorine compounds on the germination of corn

SUBSTANCE	FLUORINE	pH	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
			Polar Dent	Pickett's Dent	
	p.p.m.				*days*
Check	...	....	98.5*	99.2*	2.35
HF	100	2.88	49	70	2.25
	200	2.66	9	22	3.00
	300	2.49	0	9	4.00
	400	2.40	0	0	
NaF	100	5.47	61	72	2.00
	200	5.57	10	65	2.40
	300	5.61	12	23	2.95
	400	5.63	0	0	
0.143 *N* H_2SO_4	...	1.20	15	26	3.05
0.072 *N* H_2SO_4	...	1.43	68	67	2.10
0.072 *N* H_2SO_4 and 1 per cent CaF_2	...	1.44	0	0	
CaF_2 (solid)	...		98	99	2.30

* Average of 10 determinations of 100 seeds each. Standard deviation 1.12 and 0.75 respectively.

It should be noted that this experiment and the subsequent ones differ from the first one in that the seeds were exposed to the reagents continuously through the wick action of the "rag doll," whereas in the first experiment the seeds were exposed to the reagents only for a limited time. The results are given in table 2.

Several salient facts were brought out by the data of the second experiment. Hydrogen fluoride and sodium fluoride were both toxic to germination of corn, and their toxicity increased with concentration. Hydrogen fluoride was more toxic than sodium fluoride. These results are in accord with the findings of Bokorny (2). He attributed the toxicity to the passage of fluorine into the seed where it united with the calcium present there to form insoluble calcium

fluoride. He states: "The calcium is an integral constituent of many cell organs and probably occurs as calcium proteinate in the same." His theory is further supported by his results on the effects of potassium oxalate and oxalic acid. Both these materials proved injurious in 0.1 per cent solutions, especially the latter, which killed the seeds of cress.

Sigmund, also, found that 0.5 and 0.3 per cent solutions of tartaric acid (another chemical which forms an insoluble compound with calcium) were injurious to wheat and killed pea and rape seeds. But Sigmund obtained no germination of peas, wheat, and rape when he used solid calcium or barium fluoride.

In the present investigation, however, repeated attempts to produce injury to corn by the use of solid calcium fluoride indicated that it had no effect on the germination of corn. Seeds of corn completely embedded in the moist salt germinated readily. When 0.072 *N* H_2SO_4 was added to calcium fluoride the results were entirely different. Then the material became highly toxic, allowing no germination to take place, probably as a result of the formation of hydrogen fluoride.

The strength of the sulfuric acid used also had an effect on germination. In the jar containing 0.143 *N* H_2SO_4, not only was the percentage of germination reduced, but the roots showed signs of severe injury, barely emerging from the seed, and the shoots were also injured but to a less extent. In some cases the shoot appeared before the root, which is the reverse of the natural process. The seedlings soon became covered with mold, and it was doubtful whether they would have survived if left to grow. The 0.072 *N* H_2SO_4 treatment proved to be injurious also, but the effect was relatively much less severe. Salter and McIlvaine have shown that a nutrient solution having a pH of 2.17 allowed 26 out of 30 corn seeds to germinate. The seedlings did not flourish, however, and eventually died. A solution of pH 2.96, on the other hand, allowed nearly perfect germination (29 out of 30), but growth was retarded. Hence the acidity must be considered as a factor affecting germination.

It should also be noted that the corn seeds showed a marked variability with respect to their susceptibility to fluorine injury. For example, in the case of Pickett's Dent in the hydrogen fluoride solutions 9 per cent of the seeds were able to germinate in 300 p.p.m. of fluorine, whereas only 70 per cent of the seeds germinated in a solution containing 100 p.p.m. of fluorine. Moreover, the Polar Dent variety appeared to be more susceptible to fluorine injury than did Pickett's Dent in this experiment and also in subsequent ones.

The question arose as to whether the fluorine had a retarding effect on germination. The weighted mean (daily per cent germination times days) is very useful to express the time required for germination and is easily compared with the germination means from the other treatments. These values are found in the last column of table 2. The solutions of 100 p.p.m. of fluorine appear to have hastened germination to a slight extent, but above 200 p.p.m. a retarding action occurred. Similar effects can be observed in later experiments.

Relation of fluorine in superphosphate to germination

Because fluorine in fertilizers seemed to be a source of injury to germination and it is toxic apparently only in a soluble form, an investigation of the amount of soluble fluorine in fertilizers and its effect on germination was undertaken. Preliminary work showed that the quantity of soluble fluorine extracted from superphosphate was dependent on the quantity of water used and the length of time the fertilizer was shaken with the water. There was also a considerable difference in the amount of soluble fluorine in different superphosphates. Therefore, the following procedure was followed:

Two samples of 44 per cent superphosphate of different origin (which will hereafter be designated as A and B), and one sample of 20 per cent superphosphate were obtained from stock supplies used at this station. These samples were air dried in the laboratory and placed in suitable containers. Definite quantities of the air-dry fertilizer were weighed to 1 mgm. and added to 400 cc. of water in a 550-cc. glass bottle. These bottles were then stoppered and shaken for 6 hours in a shaking machine. Preliminary work had shown that 2 hours' shaking was sufficient to attain a maximum content of soluble fluorine in the extract, but 6 hours was allowed in order to insure complete equilibrium. After the shaking, the solutions were filtered and placed in stoppered bottles. Aliquots of the solutions were used to determine the freezing point depression by the usual Beckman thermometer method, the pH value by the quinhydrone electrode, and the soluble fluorine content by the method proposed by Willard and Winters. All these determinations were made in duplicate. Jars containing "rag dolls" were set up as before, and 200 cc. of solution was added to each. The germination test was run similarly to, and concurrently with, that of the pure fluorine compounds so that conditions of temperature, etc. were the same for both experiments. Table 3 gives the results of the tests.

Table 3 shows that superphosphate B was more toxic than the 20 per cent superphosphate, which in turn was more toxic than superphosphate A. The injury to germination produced by the solutions from the superphosphates increased with an increase in concentration of soluble fluorine. But, as might be expected, the acidity and osmotic concentration also increased with increasing amounts of fertilizer. In fact, the question might be raised whether or not the toxicity was entirely due to these last named factors. To answer this question one treatment was selected in which the values of these factors were the least. In the case of superphosphate B 1 gm. of fertilizer per 100 cc. of water gave a freezing point depression of only 0.19°C., which corresponds to an osmotic value of 2.28 atmospheres. Shive (11) obtained as good germination in solutions of 8.0 atmospheres as he did with the control cultures. The author found in further work with C. P. ammonium sulfate and potassium chloride solutions that corn seeds could germinate at much higher osmotic concentrations. Perfect germination was obtained in solutions of ammonium sulfate having a freezing point depression as high as 1.44°C. For potassium chloride the limit was still higher, 1.82°C.

The pH of the superphosphate solution under discussion happens to coincide with that of the solution used by Salter and McIlvaine on corn in which they obtained no reduction in germination. Also the slightly greater acidity of the 4-gm. solution of superphosphate A did not produce such toxicity. The fluorine content of the solution from superphosphate B was 154 p.p.m. The percentage of germination was approximately the same as that in 100 p.p.m. of fluorine as hydrogen fluoride (table 2). It must be true, therefore, that the toxicity of the fluorine in the superphosphate is modified by other factors.

TABLE 3

Per cent germination of corn in fertilizer solutions

FERTILIZER	GM. FERT. / 100 CC. H_2O	FREEZING POINT LOWERING	pH	SOLUBLE FLUORINE	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
					Polar Dent	Pickett's Dent	
		°C.		p. p. m.			days
Check	....	....	....	...	98.5	99.2	2.35
Superphosphate 20 per cent	4	0.30	2.93	117	57	87	2.80
	8	0.53	2.75	172	35	41	3.60
	12	0.74	2.65	200	7	14	3.95
	16	0.91	2.59	243	1	3	5.00
	20	1.01	2.51	270	0	1	5.00
Superphosphate A 44 per cent	4	0.52	2.94	147	91	96	2.30
	8	1.00	2.81	173	74	93	3.00
	12	1.34	2.76	214	64	68	3.85
	16	1.66	2.69	256	14	30	4.80
	20	1.98	2.66	327	3	3	4.85
Superphosphate B 44 per cent	1	0.19	2.96	154	52	69	2.65
	2	0.30	2.91	263	22	33	3.80
	3	0.43	2.87	385	12	10	3.95
	4	0.53	2.84	513	1	5	4.80
	5	0.64	2.82	618	0	5	6.80
	6	0.77	2.77	670	0	0	

A comparison of the solution of superphosphate B having 2 gm. of fertilizer per 100 cc. with that of 20 per cent superphosphate having 4 gm. per 100 cc. of water shows that the osmotic values and acidity are practically the same, and that toxicity to germination varies directly with the fluorine content. Each of these solutions, however, was less toxic than corresponding solutions of hydrogen fluoride. In the three mentioned cases the pH of the solution was higher than that of the corresponding hydrogen fluoride solutions. The solution of 20 per cent superphosphate containing 12 gm. of fertilizer per 100 cc. contained 200 p.p.m. of fluorine, and its acidity is practically the same as the hydrogen fluoride solution of that strength. The injury to germination in

this case is slightly greater than that of the hydrogen fluoride solution. It appears, then, that the toxicity is favored by high acidity. This conclusion is further supported by the fact that hydrogen fluoride proved more toxic than sodium fluoride.

Had superphosphate A been omitted from the experiment the explanation of table 3 and some later experiments would have been greatly simplified, for its injury to germination is much less than would be expected from its soluble fluorine content. There were, however, three major differences between this fertilizer and the other two fertilizers; namely, the acidity was less and the soluble fluorine was less for a given weight of available P_2O_5 than in the case of the other two fertilizers; superphosphate A contained a small amount of copper as shown by qualitative analysis of the material, whereas no trace of copper was found in the other two fertilizers. There is need of further investigation to determine the exact cause for the lower toxicity of superphosphate A.

The time required for germination in the various solutions is given in the last column of table 3. Increasing amounts of fertilizer caused greater retardation of germination. The degree of retardation was not wholly controlled by osmotic concentration, but was affected by other factors as well. No doubt the fluorine content played some part in retarding germination.

Reduction of toxicity of superphosphates by the partial removal of fluorine

Although the results of the foregoing experiments indicated that soluble fluorine was responsible for the toxicity of the investigated superphosphates to the germination of corn, an exact interpretation of the results was complicated by the influence of other factors which may have had some effect on germination. An attempt was made, therefore, to remove the soluble fluorine from the superphosphate by a method which would cause the least possible alteration of the physical and chemical properties of the superphosphate used. For this purpose the volatilization method seemed best adapted because it did not require the addition of any foreign substance except water, and fortunately the fluorine distilled over at a low temperature (120°C.), which removed only a trace of phosphoric and sulfuric acid.

Twenty-four grams of superphosphate B was accurately weighed into a 400-cc. Ehrlenmeyer flask and placed in a constant temperature oven at 135°C. $\pm$ 2°. The stoppered flask was connected to a water-cooled condenser on the outside of the oven by means of glass tubing. The superphosphate was alternately wetted and dried by the addition, through a capillary tube, of approximately 10-cc. portions of distilled water followed by several hours of drying at 135°C. The distillate was received in a suitable container. During 8 days of alternate wetting and drying 225 cc. of distillate was obtained. The hard cake of superphosphate formed by this procedure adhered to the flask so strongly that removal was not attempted, but the flask as well as its contents was broken up in a mortar, placed in a 550-cc. bottle, 400 cc. of distilled water added, shaken for 6 hours, and then filtered. The physical ap-

pearance of the fertilizer after being shaken did not seem to be different from that of the original superphosphate. The filtrate and the distillate from the superphosphate were analyzed and used for germination tests as in the preceding experiment.

Table 4 shows that not all of the soluble fluorine was removed from the superphosphate by this method, but it was greatly reduced in quantity. The reduction in soluble fluorine content was accompanied by a decrease in the toxicity of the superphosphate. The distillate from the superphosphate, on the other hand, was highly toxic to germination. This toxicity, no doubt, was due to the high concentration of fluorine which must have been in combination as hydro-fluosilicic acid because of the method used for separation. The osmotic concentration of the distillate was much too small appreciably to affect germination, and it has been shown in table 2 that 0.072 N H_2SO_4 having a pH of 1.43 was not as toxic as this distillate which had a pH of 1.89. Hence, the toxicity must have been due chiefly to the soluble fluorine present.

TABLE 4

Reduction of toxicity of superphosphates by the partial removal of fluorine

TREATMENT	GM. FERT. / 100 CC. H_2O	FREEZING POINT DEPRESSION	pH	FLUORINE	PER CENT GERMINATION	
					Polar Dent	Pickett's Dent
		°C.		p.p.m.		
Check	0	....	....	....	98.5	99.2
Superphosphate B (unheated)	6	0.77	2.77	670	0	0
Superphosphate B (heated)	6	0.59	2.22	133	56	61
Distillate from heated fertilizer	..	0.05	1.89	1015	0	0

Effect of some soils on toxicity of fertilizer solutions to germination of corn

Results obtained with solution cultures are often quite different from those obtained if the solution is added to the soil. The soil usually reduces the toxicity of solutions and may completely overcome it. An experiment was run to determine whether this was true with fluorine in fertilizers. If the solution had been added to the soil the effect of the soil on soluble fluorine could not have been determined because of lack of suitable methods for extracting the soil solution. Therefore an intermediate method was chosen.

Solutions of superphosphate B were made in the same way as the solutions given in table 3 except that 50 gm. of soil (oven-dry basis) was added for each 100 cc. of water used. The procedure for shaking, analysis, and germination was the same as before. The germination tests ran concurrently with those of the aforementioned solutions. The results are given in table 5.

The addition of soil to the solutions caused a marked reduction in the soluble fluorine content and in the acidity. This effect was accompanied by a great change in the toxicity of the fertilizer. More than twelve times as much super-

phosphate B with soil added was required to produce the degree of toxicity obtained when 1 gm. of superphosphate B was used without soil (table 3). The reduction in fluorine content, no doubt, is due to the formation of insoluble calcium fluoride, which, according to table 2, is not toxic to germinating corn. Brookston loam was more effective than Miami silt loam in reducing the soluble fluoride content of the solution. The osmotic value of the solutions was not greatly changed. The retarding effect of the solutions is shown in the last column of table 5. Six grams of superphosphate B plus soil appeared to hasten germination, but the more concentrated solutions showed a retarding influence on the germination.

TABLE 5

Effect of soils on toxicity of fertilizer solutions to germination of corn

TREATMENT	GM. 44 PER CENT FERTILIZER / 100 CC. H_2O	FREEZING POINT LOWERING	pH	FLUORINE	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
					Polar Dent	Pickett's Dent	
		°C.		*p.p.m.*			*days*
Check	..	....	....	...	98.5	99.2	2.35
44 per cent superphosphate (B)	6	0.77	2.77	670	0	0	
44 per cent superphosphate (B) plus 50 gm. Miami silt loam (pH 5.18) per 100 cc. solution	6	0.69	3.18	77	91	96	2.20
	8	0.84	3.07	116	95	99	2.55
	12	1.23	2.97	130	79	87	3.55
	16	1.52	2.88	177	38	35	4.70
	20	1.97	2.79	214	3	12	6.10
44 per cent superphosphate (B) plus 50 gm. Brookston loam (pH 7.13) per 100 cc. solution	6	0.68	3.96	12	95	96	2.05
	8	0.85	3.69	22	96	95	2.35
	12	1.23	3.43	44	95	95	3.05
	16	1.50	3.19	70	60	62	4.30
	20	1.95	3.03	116	20	39	6.35

Relation of fertilizer placement to germination of corn in pot experiments

Since the effectiveness of soil in overcoming the toxicity of the superphosphates to corn had been determined, experiments were run in the greenhouse to investigate the toxicity of the fertilizers when placed in the soil with the seed. Two-gallon glazed jars were used, containing soil made up to definite moisture contents; 7,000 gm. of air-dry soil was used in the case of Brookston loam and 8,000 gm. in the case of Miami and Fox soils.

The rate of application of fertilizer was based upon a hill application in a circle 3 inches in diameter with the corn planted in check rows 3½ feet apart. By this method only 1/250 of the total area is covered. Hence, an application of 25 pounds per acre in the hill equals 25 times 250 or 6,250 pounds per acre

broadcast for the area covered. The fertilizer was applied with large salt shakers, the entire cross section of the soil being covered at the broadcast rates noted. Fifty seeds were planted per jar at a depth of 2½ inches. The jars were made up to weight every other day by adding water to the lower soil through a glass tube placed through the center of the soil column.

An experiment was conducted to determine what effect placing the embryo against the fertilizer would have upon germination as compared to the same treatment with the endosperm toward the fertilizer. Corn was planted on Miami silt loam at 10 per cent moisture content, 20 per cent superphosphate being used in contact with the seed. The placements and results are given in table 6.

Since the water was added to the lower part of the soil column the movement of moisture in the soil was predominantly upward as it would be in the field if no rains occurred during the time for germination. Under these conditions the fertilizer below the seed was injurious to germination of corn regardless of whether the fertilizer was in contact with the embryo or the endosperm. The fertilizer above the seed produced greater injury when the embryo came in contact with the fertilizer than when the endosperm did. Plate 1 shows these jars 12 days after the seed was planted. Similar results with regard to facing the embryo of the corn kernel toward or away from the fertilizer were obtained by Coe, using Ammo-Phos in contact with the seed. The experiment indicates that when the diffusion of the fertilizer takes place in a direction away from the seed a few millimeters separation of seed and fertilizer may make a profound difference in the percentage of germination, for when the fertilizer was above the seed in contact with the endosperm, germination was much better than when the embryo was exposed to the fertilizer.

TABLE 6

Relation of fertilizer placement to germination of corn in pot experiments

PLACEMENT OF FERTILIZER	PER CENT GERMINATION	
	12,500 lbs./A.	25,000 lbs./A.
No treatment	98	100
Fertilizer below seed in contact with embryo	18	0
Fertilizer below seed in contact with endosperm	14	12
Fertilizer above seed in contact with embryo	14	10
Fertilizer above seed in contact with endosperm	82	42

Influence of soil moisture on toxicity of fertilizers to germination of corn

Truog and his co-workers (13) found that the injury to germination of corn caused by a complete fertilizer varied inversely with the moisture content of the soil. An experiment was conducted to determine whether the same was

true for superphosphates. Jars of Miami silt loam were made up to definite moisture content. The fertilizer was applied beneath the seed in contact with the embryo. Superphosphates A and B and 20 per cent superphosphate were included in this experiment. The amount of fertilizer used in the case of the more concentrated carriers was such that an equivalent amount of available P_2O_5 was used in all three sets of pots. Watering was accomplished in the same manner as before. Daily counts were made of the number of plants emerging from the soil and from these the weighted mean for time of emergence was calculated. Table 7 contains these results.

The data show that germination was both retarded and reduced by a decrease in soil moisture content. Similar results were obtained by using Brookston loam at 20 per cent and 15 per cent soil moisture content. The nature of

TABLE 7

Influence of soil moisture on toxicity of fertilizers to germination of corn

FERTILIZER	RATE OF APPLICATION	PER CENT GERMINATION		TIME REQUIRED FOR EMERGENCE	
		15 per cent soil moisture	10 per cent soil moisture	15 per cent soil moisture	10 per cent soil moisture
	lbs./A.			*days*	*days*
Check	.....	98	100	5.0	5.6
Superphosphate 20 per cent	6,250	80	58	7.0	9.6
	12,500	50	14	8.6	10.0
	25,000	12	0	9.7	....
Superphosphate A 44 per cent	2,841	96	96	6.3	7.2
	5,682	84	80	8.2	8.7
	11,364	52	12	10.0	11.2
Superphosphate B 44 per cent	2,841	86	80	5.8	7.8
	5,682	44	48	8.4	8.8
	11,364	18	0	8.8	....

the experiment precluded reliable chemical analysis of the solution coming in contact with the seed, but from the laboratory results certain suppositions seem justified. The lower moisture content of the soil probably resulted in a higher concentration of soluble material, including fluorine, at the surface of the seed. Also, no doubt, there was a slower rate of reaction between fertilizer and soil at the lower moisture content than at the higher one, for diffusion in the soil was probably slower. The combination of the two conditions named should have favored a higher soluble fluorine content as well as a higher osmotic concentration in the dryer soil, resulting in retardation and inhibition of growth.

Effect of mixing superphosphates with soils vs. direct contact with seed on germination

An experiment was set up to determine the effect on germination of mixing the superphosphates with the soil as compared to the placement of the same in direct contact with the seed. The jars for the direct contact treatments were made up as before, the fertilizer being placed below the seed in contact with the embryo. In the jars where mixing of fertilizer with the soil was desired, 1 kgm. of air-dry soil was mixed with the fertilizer, brought to the desired

TABLE 8

Effect of mixing fertilizer with soils on the toxicity of fertilizers to the germination of corn

FERTILIZER	RATE OF APPLICATION	PER CENT GERMINATION IN BROOKSTON LOAM		PER CENT GERMINATION IN MIAMI SILT LOAM		PER CENT GERMINATION IN FOX SANDY LOAM	
		Contact	Mixed	Contact	Mixed	Mixed limed	Mixed not limed
	lbs./A.						
Check	.....	100	98	98	100	98	98
Superphosphate 20 per cent	6,250	90	...	80	...	...	...
	12,500	46	98	50	98	100	100
	25,000	0	98	12	98	100	100
	50,000	0	100	0	100	100	100
	100,000	..	100	..	98	98	92
Superphosphate 44 per cent A	2,841	98	...	96	...	...	...
	5,682	78	96	84	98	98	100
	11,364	16	100	52	100	100	98
	22,727	0	100	0	100	100	100
	45,454	..	100	..	94	8	6
Superphosphate 44 per cent B	2,841	74	...	86	...	...	...
	5,682	56	100	44	98	100	100
	11,364	6	98	18	96	98	98
	22,727	0	98	0	98	100	98
	45,454	..	94	..	92	2	0
pH of soil	.....	7.13		5.18		6.48	5.10

moisture content, and placed in a layer over untreated moist soil. In this layer the corn kernels were planted. The layer of fertilized soil was approximately 1 inch thick and was covered by a layer of unfertilized soil. Three soils were used—Brookston loam at 20 per cent moisture content, Miami silt loam at 15 per cent moisture content, and Fox sandy loam at 12 per cent moisture content. The moisture content was approximately one-half of the moisture-holding capacity. The results of the experiment are recorded in table 8.

By mixing the fertilizer with the soil the toxicity of the fertilizer was almost

completely overcome except in the case of the heaviest application of the 44 per cent superphosphates on Fox sandy loam. More than sixteen times as much fertilizer could be applied without reduction in germination when the fertilizer was mixed with the soil as when it was used in direct contact with the seed. When the superphosphates were in direct contact with the seed the reaction between soil and fertilizer was not complete enough to overcome its toxicity.

It was supposed that the soil type might play an important part in the ability to overcome toxicity. Except for the heaviest application on Fox sandy loam, the results are rather unconvincing. The direct contact results indicate that reaction between soil and fertilizer was not sufficient to produce significant differences, whereas the reaction between the mixed soil and fertilizer was great enough to overcome toxicity in the majority of the cases.

The lower toxicity of superphosphate A is again brought out in the direct contact treatments of table 8. The difference between the toxicity of superphosphate B and 20 per cent superphosphate, however, was diminished as compared to the toxicity produced as shown in table 3. This can be accounted for by the fact that in the case of 20 per cent superphosphate, a greater weight of dry fertilizer was used which must have reduced the moisture content in the immediate vicinity of the seed.

Increased retardation of emergence of corn occurred with increasing amounts of fertilizer. The seed in soil mixed with fertilizer came up 1.3 days earlier as an average than when fertilizer was applied at the same rate in direct contact with the seed.

Toxicity of fertilizers to germination of corn in field experiments

The final experiment with respect to toxicity of superphosphates was conducted in the field. The fertilizer was applied in the hill in a circle 3 inches in diameter, and the seeds were planted in direct contact with the fertilizer. Amounts of the 44 per cent fertilizers equivalent in P_2O_5 content to the amounts applied in the 20 per cent superphosphate applications were used. For each treatment ten hills were planted with five kernels of corn per hill. At the time of planting, samples of soil were taken on which moisture content and pH were determined. Emergence counts were made at 12 and 21 days from date of planting. The final emergence is given in table 9.

The results of the greenhouse tests are confirmed by the field tests. There was a marked difference produced by facing the embryo toward or away from the fertilizer. The low moisture content probably accounts for the lower percentage of germination on Hillsdale sandy loam. Superphosphate A proved less toxic than superphosphate B or superphosphate 20 per cent. The percentage of germination, however, was much higher in the field experiment than in the greenhouse experiment. This may be due, in part, to the fact that only a circular area 3 inches in diameter was covered with fertilizer, whereas in the jars in the greenhouse the entire cross section of the jar was covered. The

plants that grew showed a marked tendency to send the roots through the soil beyond the fertilizer rather than through the fertilizer. Further comparison of percentage of germination between the greenhouse and field tests scarcely seems justified because different soils having different moisture contents were used and the climatic environment was entirely different.

TABLE 9

Toxicity of fertilizers to germination of corn in field experiments

OBSERVATION NUMBER	FERTILIZER	RATE OF APPLICATION	PER CENT GERMINATION		
			Fertilizer placed above seed vs. endosperm	Fertilizer placed below seed vs. embryo	Fertilizer placed below seed vs. embryo
		lbs./A.			
1	Check	....	98	92	94
2	Superphosphate 20 per cent	50	86	58	54
3		100	68	20	14
4		200	60	8	0
5	Superphosphate 44 per cent A	=50	96	84	88
6		=100	98	58	26
7		=200	86	24	24
8	Superphosphate 44 per cent B	=50	88	46	34
9		=100	82	14	6
10		=200	34	6	0
Soil type			Hillsdale loam	Hillsdale loam	Hillsdale sandy loam
Soil moisture		*per cent*	20.3	21.3	10.4
pH			7.86	7.81	7.84

SUMMARY

In this investigation, laboratory, greenhouse, and field experiments have shown that the superphosphates investigated were capable of exerting a toxic influence on the germination of corn as a result of their content of soluble fluorine. The soils used in the experiment were effective in reducing or overcoming the toxicity provided sufficient reaction took place between the soil and the fertilizer. These two facts furnish a satisfactory explanation of the high toxicity of superphosphates when placed in direct contact with the seed and also of the reduction of this toxicity when the superphosphates were mixed with the soil. Corn was found to be very variable in its susceptibility to fluorine injury. The facing of the embryo of the seed toward the superphosphate resulted in greater reduction in germination of corn than when the position of the kernel was reversed, provided the fertilizer diffused away from the seed. There was considerable difference in the amount of injury produced by the three superphosphates investigated. Further research is needed to determine the effects of nitrogen and potash carriers on the soluble fluorine content and on the toxicity of the superphosphates in mixtures of these fertilizers.

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PLATE 1

Relation of Fertilizer Placement to Germination of Corn

20 per cent superphosphate on Miami silt loam at 10 per cent moisture content. Fertilizer was placed above the seed.

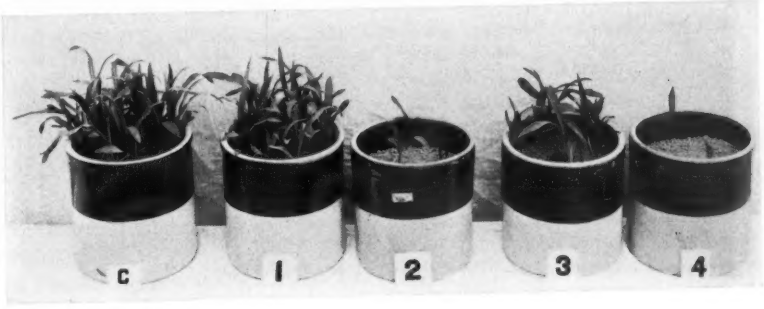
C—No fertilizer.

1—12,500 lbs./acre in contact with endosperm.

2—12,500 lbs./acre in contact with embryo.

3—25,000 lbs./acre in contact with endosperm.

4—25,000 lbs./acre in contact with embryo.



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ISOLATION OF SOME BACTERIA WHICH OXIDIZE THIOSULFATE[1]

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The sulfur bacteria have several physiological characteristics in common. They are autotrophic (5, 59, 66, 74) and make use of elemental sulfur or some inorganic incompletely oxidized sulfur compound as a source of energy. During their development, this specific sulfur substance becomes more completely oxidized, and the energy released by the reaction enables the bacteria to reduce dissolved carbon dioxide. The various organic constituents of the bacterial cells are constructed from these reduction products.

The members of this physiological group of bacteria are extremely diverse, particularly in morphological characteristics and to a surprising degree physiologically. In Bergey's Manual (9), the sulfur bacteria are classified under two such different orders as *Eubacteriales* and *Thiobacteriales*. According to van Niel (64), this latter order should be split into two orders, the *Rhodobacteria*—purple bacteria; and *Thiobacteria*—colorless sulfur bacteria. An additional group of pigmented forms is encountered in the green bacteria which appear to be most closely related to the purple forms in respect to their utilization of sulfide as a source of energy. The purple and green bacteria are distinctive physiologically from all other sulfur bacteria by their requirement for radiant energy in the form of light which is utilized through the agency of their pigments. The fact that they are anaerobic and that the purple bacteria are facultative autotrophs are further distinctive features (45, 64, 65). Present information suggests the likelihood that the physiological utilization of the specific inorganic sulfur substances is much the same by members of both the colorless *Thiobacteriales* and the *Thiobacillus* members of the *Eubacteriales* in spite of the pronounced differences in morphology of these two groups of organisms. Even the accumulation of sulfur within the cells of the larger *Thiobacteriales* has lost its early significance with the demonstration by van Niel (64) that, when sulfur is formed, it occurs within the cells of only the larger individuals and outside the small cells.

The sulfur bacteria were first characterized by Winogradsky (73) from examination of large forms, both pigmented and colorless. This characterization, having preceded the discovery of the group of small sulfur bacteria, probably led Omeliansky to distinguish as true sulfur bacteria only those larger forms that generally showed sulfur globules within the cells (47). In discussing the differences between Nathansohn's culture and the organisms obtained by Winogradsky, Omeliansky wrote, in 1904 (47, p. 239): "Ihre Oxydationskraft ist bedeutend schwächer als bei diesen, da sie nur imstande sind, Thiosulfate zu Tetrathionsäure und Schwefelsäure zu oxydieren. Auch Morphologisch unterschieden sie sich scharf von den echten Schwefelbakterien, da bei ihnen niemals intracelluläre Ausscheidung von Schwefel stattfindet. Man ist also wohl berechtigt, diese Gruppe von den echten Schwefelbakterien abzuscheiden und mit einem besonderen Namen zu belegen. Wir schlagen dafür 'Thionsäurebakterien' vor." During the years which have since ensued it has been shown that some members of the genus *Thiobacillus* are able to oxidize sulfur and other sulfur compounds fully as energetically as is reported for at least some of the larger sulfur bacteria. As an

[1] Journal Series paper of New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

example we might cite a case of oxidation by *Th. thiooxidans*. When large amounts of elementary sulfur were initially present in the culture solution (25 gm. sulfur to 100 cc. of solution) about 500 mgm. of sulfur were oxidized by this bacterium in 10 days (70). Typical purple and green sulfur bacteria studied by van Niel (64) oxidized from about 5 to 25 mgm. of H_2S in 2 weeks. However, it seems likely that oxidation of sulfide by species of *Thiobacillus* is less energetic and less representative of the group than with the larger sulfur bacteria. Intensity of oxidizing capacity does not seem to be a suitable criterion for separating the two groups of organisms. Even the presence of sulfur within the cells has lost its earlier significance.

A very limited conception of the sulfur bacteria is expressed in the recent book by Ellis. He states (14, p. 3), "The term sulphur bacteria embraces only those organisms which oxidize the H_2S to S, store the latter temporarily in their bodies, and then oxidize it to SO_4." Such a viewpoint is not only incompatible with our knowledge of the physiology of the *Thiobacillus* species, but it also fails to typify the higher sulfur bacteria.

There seems to be no valid reason why all of the bacteria which can utilize inorganic sulfur materials as the specific energy sources while developing as autotrophs, should not be classified as true sulfur bacteria. Morphological features certainly should not be the major criteria for determining whether or not a bacterium belongs to a specific physiological group.

Ellis also misappropriated the genus *Thiobacillus* for some members of the larger sulfur bacteria. He states (14, p. 130), "The former word (*Thiobacillus*), however, is frequently used loosely to indicate certain thionic acid bacteria, and in particular *Thiobacillus thiooxidans* which in the strict sense is not one of the sulphur bacteria. Up to the present, the term *Thiobacillus* has been used in the publications of American writers as a convenient catalogue name, devoid of any phyletic significance." In such statements he shows an extreme lack of appreciation of the activities of the *Thiobacillus* species. Far from being used loosely in referring to certain bacteria, the term *Thiobacillus* has been applied to a very specific group of bacteria since its introduction in 1904 by Beijerinck (7, 8), and this group includes only autotrophic sulfur bacteria of the order Eubacteriales. To state that *Th. thiooxidans* is not a sulfur bacterium clearly is an admission of misconception. This organism is a very typical bacterium which is strictly autotrophic and is able to obtain energy for its development only from elemental sulfur, thiosulfate, or similar incompletely oxidized inorganic sulfur compounds.

Since Omeliansky first applied the term "Thionsäurebakterien" to the group of bacteria now known as members of the genus *Thiobacillus*, it has been customary to consider them under this title. This implies certain ideas which are incompatible with the facts. Although bacteria of this group all appear to be able to oxidize thiosulfate, their activity is not confined to this substance and some of them also oxidize elemental sulfur and certain polythionates. On the other hand, the purple sulfur bacteria also oxidize thiosulfate in addition to sulfur, sulfide, and sulfite (64). Furthermore, thiosulfate appears to be fully as satisfactory as sulfide for the purple bacteria, since it can be used in higher concentrations than sulfide without injury to the bacteria. In view of these facts the terms "Thiosulfate Bacteria" and "Thionsäurebakterien" are incongruous in their present application and it would seem wise to discontinue their use.

It is with some members of the genus *Thiobacillus* that the studies of this and some following reports are primarily concerned. More specifically, the investigations deal with the characterization of some of these forms from the nature of their growth upon media containing thiosulfate. Hasty judgment might lead one to conclude that the examination of soils for organisms capable of oxidizing thiosulfate would lead to results which have little or no application in natural phenomena. Although thiosulfate is seldom applied directly to soils it may reach the soil from various sources. It appears in some spring waters as a product of the incomplete oxidation of sulfide (12). It may arise in a similar manner in natural black mud (5). Of greater significance is the probability of its formation as an intermediate

product during the oxidation of elemental sulfur in soils. The results of Guittonneau and Keilling clearly show that numerous heterotrophic organisms are able to oxidize sulfur to thiosulfate both in soils and in solution media (19–28). Further oxidation appears to be effected by the sulfur bacteria. Elemental sulfur is applied to soils for correcting soil reaction, and larger quantities reach the soil as residues from dusting and spraying. Guittonneau and Keilling calculate that at least 100,000 tons of sulfur are used each year to treat the French vineyards as a fungicide. In this country increasing amounts of sulfur are being used in the control of parasitic fungi and insects. As much as 320 pounds an acre are applied in a single season in some commercial apple orchards (42). Some thiosulfate may be produced during the transformation of sulfur which is contained in the plant and animal residues that reach the soil.

The importance of bacteria capable of oxidizing thiosulfate assumes broader significance when it is realized that many, or possibly most, of them are also able to oxidize other inorganic sulfur substances which occur in natural environments in greater quantities than thiosulfate. Furthermore, it is particularly desirable to isolate the organisms in thiosulfate media, since in many instances there is better development than upon other sulfur compounds.

In spite of the fact that a comparatively large number of reports have dealt with members of the genus *Thiobacillus*, in few instances has sufficient information been presented concerning the morphology and physiology of the bacteria to enable one to determine whether or not new species were being considered. Although there are but four species of *Thiobacillus* listed in Bergey's Manual, no less than 12 species names have been published, and an equal additional number of species could be distinguished in the list of organisms already described in the literature, if minor differences justified the creation of new species.

DISTRIBUTION

The first detailed study of these bacteria is generally credited to Nathansohn (46). By his culture obtained from sea water at Naples, the thiosulfate was decomposed to sulfate and elemental sulfur. The following reaction was proposed to explain the reaction:

$$2\ Na_2S_2O_3 + O_2 = 2\ Na_2SO_4 + 2\ S \qquad (A)$$

Since incompletely oxidized sulfur compounds appeared in the medium he finally concluded that the important reaction was:

$$6\ Na_2S_2O_3 + 5\ O_2 = 2\ Na_2S_4O_6 + 4\ Na_2SO_4 \qquad (B)$$

The precipitated sulfur was believed to arise from secondary reactions. If his conclusions were correct, he dealt with a transformation that has not since been described. It is conceivable that his culture was impure.

Beijerinck studied similar organisms obtained from fresh water, canal mud, and salt water (7, 8). Reaction (*A*) was believed to best represent the transformation. The name *Thiobacillus thioparus* was applied to the bacterium. Jacobsen isolated the same organism from soil, ditch water, sewage, and sea water and studied its oxidation of sulfur (33) and sulfide (34).

Organisms similar to *Th. thioparus* were obtained by Issatschenko, from the sea mud of the Arctic Ocean (29); by Brenner from marine mud of Kiel, rich in H_2S (10); and by Guittonneau from soil in France (20, 21, 22, 24).

Cultures which may have been identical with *Th. thioparus* were obtained by Waksman et al. from an alkali soil from California (71); by Klein and Limberger from ditch mud, composts, beach sand, and various soils of Germany (38); by Klein and Steiner in the mud and water of Lake Lunz in Germany (39); by Szimmat in garden soil, alder bog, and moor soil of East Prussia (60); and by Wudtke in 29 of 85 soils of waste land near the Baltic Sea (75). Organisms effecting the oxidation of thiosulfate in much the same manner as *Th. thioparus* were detected by Joffe in nine New Jersey soils and in eight from Oregon. The only soils not indicating presence of the organism were soils which had become very acid subsequent to the addition of sulfur (36).

These facts seem to indicate that *Th. thioparus*, or species closely related to it, are widely distributed in nature.

Beijerinck studied a second organism obtained from canal water, mud, and salt water. It developed in the absence of free oxygen and oxidized thiosulfate or sulfur, causing the reduction of nitrate to gaseous nitrogen (7, 8).

$$6\ KNO_3 + 5\ S + 2\ CaCO_3 = 3\ K_2SO_4 + 2\ CaSO_4 + 2\ CO_2 + 3\ N_2 \qquad (C)$$

This organism was named *Th. denitrificans*. It was believed to be a facultative autotroph, but from the results of Lieske (41) and Gehring (17), it seems likely that it is strictly autotrophic. Lieske obtained his cultures from mud of the Leipzig Botanical Gardens. Gehring, working at Göttingen, found the organism very generally distributed and detected it in cultivated soils, earth composts, beech forest soils, peats, and mud. More recently, Tjulpanova-Mossevitch recovered similar bacteria from two fresh water and four salt water basins in Russia (61). These cultures were facultative anaerobes and facultative autotrophs! *Th. denitrificans* appears to be widely distributed but, because of difficulties of isolation, has been less generally recognized than *Th. thioparus*.

A third species was isolated from sulfur-soil-phosphate composts by Waksman and Joffe (67, 68). This organism, named *Th. thiooxidans*, oxidizes thiosulfate and sulfur to sulfate and develops under extremely acid conditions.[3]

$$2\ S + 3\ O_2 + 2\ H_2O = 2\ H_2SO_4 \qquad (D)$$

Brown obtained a similar organism from garden soil, activated sludge, and soil-sulfur compost (11). Joffe recovered the organism only from soils which had been treated with sulfur (36). The bacterium has more recently been found by Jensen in a dried up sea bed in Denmark (35), by Drewes in a bog about Kiel (13), and by Waksman in waters of a Mexican sulfur spring (6). *Th. thiooxidans* was detected in only 1 of 55 peat soils of Minnesota studied by Skinner and Nygard, and this soil had previously been fertilized with flowers of sulfur (56). In a garden soil, a moor, and an alder bog, Szimmat found organisms which were believed to be *Th. thiooxidans* (60). Wüdtke claims to have encountered the bacterium in 22 of 85 soils in East Prussia (75). Wilson and Higbee reported that organisms of similar character occur in most of the important mineral soils of New York State (72). In these last three reports the results are too meagre to indicate whether or not *Th. thiooxidans* occurred in the soil populations.

Ayyar, Perumal, and Norris working in India obtained a culture from activated sludge which reacted much the same as *Th. thiooxidans* (2, 3, 4). One of the cultures isolated by Aquino from a soil in the Philippines was believed to be *Th. thiooxidans* (1). Since the organisms of both Aquino and Ayyar developed upon organic media, they are not identical with *Th. thiooxidans*. It is possible that they were using mixed cultures.

Rountree isolated from five different soils of South Australia an organism which reacted much the same as *Th. thiooxidans* (49). It differed from *Th. thiooxidans* in size and in the range of hydrogen-ion concentrations over which it developed. Emoto described and named four species which were obtained from thermal sulfur springs in Japan (15, 16). These were called *Th. thermitanus*, *Th. lobatus*, *Th. crenatus*, *Th. umbonatus*. The only features which distinguished one of these species from another or from *Th. thiooxidans* were differences in size of cells and minor variations in appearance of colonies on solid media.

Trautwein obtained cultures from sewage purification plants, the River Tauber, and soils. They were described as facultative autotrophs which formed sulfate and polythionates from thiosulfate (62, 63).

$$4\ Na_2S_2O_3 + 6\ O_2 = 4\ Na_2S_2O_6 \qquad (E)$$

$$3\ Na_2S_2O_3 + 5\ O = Na_2S_4O_6 + 2\ Na_2SO_4 \qquad (F)$$

These bacteria were distinctly different from any of the forms previously described as sulfur bacteria. They more closely resembled *Bacterium denitrificans* Stutzer and Burri (L & N).

Halophilic cultures closely resembling *Th. thioparus* have been obtained from various salt seas and lakes in Russia and Siberia. Issatschenko and Salimowskaja describe two species and one variety: *Th. Beijerinckii*, *Th. Nathansonii*, and *Th. Beijerinckii* var. *Jacobsenii* (31,

[3] For complete list of studies of this organism see references 57, 58.

32). They differ somewhat in cell size and tolerance to salt concentrations. Such organisms were detected in all 10 of the salt seas studied, in the Arctic Ocean, Tambukan sulfate lake, and soda lakes of Siberia (30, 40).

Other halophilic forms similar to those obtained by Issatschenko were isolated by Saslawsky and associates (51–55). One form came from the Kujalnitzky Liman and a second from the Ssuchoi Liman, the second oxidizing the thiosulfate completely to sulfate and the first leaving some unoxidized material.

Several additional cultures have been obtained which do not seem to be identical with any of those mentioned in the foregoing. Salimowskaja's organism was isolated from the river Fontanka (50). It developed much the same as *Th. thioparus* except that it was favored by a moderate acid reaction and brought about an increase in pH of the cultural medium during growth. Lockett studied the oxidation of thiosulfate by microörganisms in systems much like bacterial sewage filters (43, 44). Some bacteria recovered from the filters decomposed thiosulfate to sulfate in media containing gelatin or Rochelle salt, but failed to grow on the common laboratory media in the absence of thiosulfate.

A very tiny coccoid bacterium which was able to break down thiosulfate to sulfur and sulfate was recovered by Paine, Linggood, Schlimmer, and Thrupp from corroding walls and building stones (48). This organism was believed to play an important rôle in the disintegration of the masonry.

Gicklhorn named and described two organisms (*Bacterium cristalliferum* and *Bacterium retiformans*) which were developing in sulfide water (18). They were not obtained in pure culture, and no determination was made of their action upon sulfur compounds, but it was believed that they were sulfur bacteria.

Included in the assortment of transformations which have been ascribed to sulfur bacteria is the following, proposed by Beijerinck in 1904 but receiving no further attention. The organism was an anaerobic bacterium, thought to belong to the genus *Thiobacillus*, able to produce sulfide at the expense of sulfite and subsequently oxidize the sulfide to sulfur. Organic matter was required by the organism (7).

$$C_3H_5NaO_3 + 2\ Na_2SO_3 = NaHCO_3 + 2\ Na_2CO_3 + 2\ H_2S \qquad (G)$$

$$2\ H_2S + O_2 = 2\ H_2O + S_2 \qquad (H)$$

The evidence in the various reports clearly indicates that sulfur bacteria of the genus *Thiobacillus* are widely distributed. They occur in most soils, upon rocks, in salt water and fresh water, in sewage, and in mineral springs. Organisms capable of oxidizing thiosulfate may even be sufficiently abundant as laboratory contaminants to cause the decomposition of the thiosulfate solutions which are used as chemical reagents (37).

EXPERIMENTAL

For detecting the presence of organisms oxidizing the sulfur materials, the media shown in table 1 were used. The thiosulfate and bicarbonate, where used, were sterilized separately, as was also the ammonium sulfate for media II, III, and IV. The media were used in 100-cc. portions in 250-cc. Erlenmeyer flasks.

Soils were collected with aseptic precautions. Each of three flasks received 100-gm. portions of each soil. One portion was untreated; the second received 2 gm. sterile sulfur, and the third, 1 gm. sterile sodium thiosulfate. The moisture content was kept favorable during the 3 weeks of incubation at 28°C. Suspensions containing 0.5 gm. soil were then inoculated into flasks of thiosulfate and sulfur media.

Seven samples of soil (all black clay loams) were obtained from the fruit farm of the University of Minnesota. Nine other samples were obtained from Wis-

consin, numbers 8–13 being clay or clay loams and numbers 14–16 being sandy loams. These all supported field crops or grass. The soils were close to neutrality in reaction, varying between pH 6.1 and 7.1. As a result of incubation with sulfur the reactions dropped to pH 1.8–4.3. There was no appreciable change in reaction in any of the soils due to the presence of thiosulfate.

None of the soils caused growth, change in reaction, or sulfur oxidation in the acid sulfur solution medium (I). Enrichment of the soil with sulfur or thiosulfate previous to inoculation failed to effect the development of organisms in this medium even though the soil reaction had become very acid during the preliminary incubation. Oxidation of thiosulfate was rapid with most of the soils upon inoculation into medium II (table 2). The pretreatment with sulfur or thiosulfate failed to favor the process; in fact, somewhat slower oxidation was obtained from treated soils 1–7. The characteristics of growth were

TABLE 1

Media used for cultivating organisms upon sulfur materials

CONSTITUENTS	I	II	III	IV
	For Th. thiooxidans	For thiosulfate decomposers		
Distilled water	100.0 cc.	100.0 cc.	100.0 cc.	.....
Tap water	.....	.....	.....	100.0 cc.
Sulfur	1.0 gm.	.....	.....	.....
$Na_2S_2O_3 \cdot 5H_2O$	.....	0.5 gm.	0.5 gm.	1.0 gm.
$(NH_4)_2SO_4$	0.04 gm.	0.04 gm.	0.02 gm.	0.01 gm.
KH_2PO_4	0.4 gm.	.....	.....	.....
K_2HPO_4	.....	0.4 gm.	0.2 gm.	0.2 gm.
$CaCl_2$	0.025 gm.	0.025 gm.	0.025 gm.	0.01 gm.
$MgSO_4 \cdot 7H_2O$	0.05 gm.	0.05 gm.	0.02 gm.	0.01 gm.
$MnSO_4$	.....	.....	.....	0.002 gm.
$FeSO_4$	0.001 gm.	0.001 gm.	0.001 gm.	0.002 gm.
$NaHCO_3$	.....	.....	0.05 gm.	.....
pH	4.8	7.0	8.5	7.8

diverse. In some cases the medium was turbid; in others, sulfur precipitated on the liquid surface or upon the wall of the flasks; and in still other cultures, thin membranes, free from sulfur, appeared on the surface. Combinations of these effects were also noted. As growth progressed the reactions of the media became somewhat acid in all cases although varying in degree among the cultures. Transfers were made to fresh media, in which the thiosulfate was again rapidly oxidized. Some of the cultures were plated upon an agar medium containing thiosulfate, and colonies were recovered which in some cases were impregnated with sulfur and in other instances appeared small and moist like drops of dew. Almost invariably there were clear zones about the developing masses of bacteria, suggesting the formation of acid during oxidation of the thiosulfate. These cultures decomposed thiosulfate upon reinoculation into the solution medium.

Thirteen samples of soil materials were gathered from the farm of the N. J. Agricultural Experiment Station (see table 3) and inoculated, without preliminary treatment, into the acid sulfur solution medium. These soils varied considerably in texture, reaction, and vegetation. Only two of the samples gave growth and sulfur oxidation in medium I. One of these (soil 29) had

TABLE 2

Oxidation of thiosulfate in solution medium II following soil inoculation

PRETREATMENT OF SOIL	SOIL NUMBER	THIOSULFATE REMAINING IN 5 CC. OF MEDIUM			SOIL NUMBER	THIOSULFATE REMAINING IN 5 CC. OF MEDIUM		
		10 days	15 days	30 days		10 days	15 days	30 days
		mgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*
Untreated	Control	19.2	19.2	21.3	Control	19.2	19.2	19.2
	1	0	0	0	8	13.8	....	0
	2	0	0	0	9	19.2	....	11.9
	3	0	0	0	10	2.1	....	0
	4	0	0	0	11	14.9	....	14.5
	5	0	0	0	12	0	....	0
	6	0	0	0	13	19.2	....	1.3
	7	0	0	0	14	19.2	....	17.7
					15	9.6	....	3.1
					16	9.6	....	1.9
2 per cent s	1	17.3	0	0	8	13.6	0	0
	2	16.0	18.0	18.0	9	14.9	14.1	14.9
	3	18.5	19.0	20.3	10	....	....	....
	4	18.0	19.2	20.9	11	13.0	0	0
	5	17.7	15.3	0	12	15.3	13.4	9.2
	6	0.4	0	0	13	16.4	13.6	9.8
	7	5.1	0	0	14	4.3	0	0
					15	15.5	15.1	16.3
					16	0	0	0
1 per cent $Na_2S_2O_3$	1	6.0	0	0	8	16.8	14.1	0
	2	18.1	18.7	18.3	9	18.7	19.2	22.4
	3	13.8	1.7	0	10	18.1	10.7	0
	4	14.9	0	0	11	17.5	15.8	16.8
	5	0	0	0	12	16.2	0	0
	6	12.8	0	0	13	14.5	8.2	0.6
	7	0	0	0	14	17.5	16.1	0
					15	18.1	17.6	16.8
					16	12.1	0	0

received a heavy application of sulfur and rock phosphate and had been inoculated with *Th. thiooxidans* about 10 years previous to the sampling. There still remained a considerable quantity of unoxidized sulfur. The extremely acid condition (pH 2.2) is clear evidence of extensive sulfur oxidation. Growth from this soil was active and typical for *Th. thiooxidans*. Evidently,

TABLE 3

Decomposition of thiosulfate in solution media following soil inoculation

SOIL NUMBER	FIELD TREATMENT*	pH OF SOIL	THIOSULFATE			
			32 days after first addition		Second addition of thiosulfate 11 days after addition	
			Found	Decomposed	Found	Decomposed
			mgm.	*mgm.*	*mgm.*	*mgm.*
Blank	.....	...	20.1	....	22.6	....
17	(7A) Untreated	4.7	16.6	3.5	15.0	7.6
			15.8	4.3	18.6	4.0
18	(7B) Lime	6.9	19.0	1.1	....	....
			15.8	4.3	17.8	4.8
19	(5A) Minerals, manure	5.8	1.6	18.5	0	22.6
			5.1	15.0	10.7	11.9
20	(5B) Minerals, manure, lime	7.1	0	20.1	23.3	0
			0.8	19.3	10.7	11.9
21	(9A) Minerals, $NaNO_3$	5.5	17.8	2.3	....	....
			18.2	1.9	....	....
22	(9B) Minerals, $NaNO_3$, lime	7.2	16.6	3.5	....	....
			0	20.1	21.7	0.9
23	(11A) Minerals, $(NH_4)_2SO_4$	4.1	18.6	1.5	....	....
			17.0	3.1	11.1	11.5
24	(11B) Minerals, $(NH_4)_2SO_4$, lime	6.0	15.0	5.1	5.9	16.7
			15.0	5.1	6.3	16.3
25	Vegetable crops, sandy loam	7.0	15.4	4.7	13.8	8.8
			15.8	4.3	20.5	2.1
26	Pasture, clay loam	7.0	15.8	4.3	15.8	6.8
			5.5	14.6	19.8	2.8
27	Hardwood forest, A_0 horizon	4.6	9.9	10.2	16.6	6.0
			17.8	2.3	....	....
28	Hardwood forest, A_1 horizon, clay	4.6	19.0	1.1	....	....
			19.0	1.1	....	....
29	Barren sulfur treated loam	2.2	....	....	....	....

* Soils 17–24 are from experimental plots receiving different fertilizer treatments for over 20 years. The soil is a sandy loam supporting field crops in rotation.

this organism will persist for a long time, if not indefinitely, in soils under natural conditions if elemental sulfur is available. The development of an extremely acid condition appears to have no injurious effect in soil. It has previously been found to exert very slight action in solution media (57).

The other sample which gave growth was number 27, which was composed of decomposing litter of a hardwood forest. Growth in this case was somewhat slower than by the usual cultures of *Th. thiooxidans* although the reaction was brought below pH 1.0 by growth in some of the flasks of medium I to which the culture was transferred. This culture was cultivated with monthly transfers for more than 2 years before being discarded.

These results again emphasize that *Th. thiooxidans* is not generally readily recovered from soils or other natural materials which are not supplied with elemental sulfur or similar inorganic sulfur materials.

Medium III which was used for these soils is similar in composition to that used by Beijerinck for *Th. thioparus* (7, 8). The soils were inoculated in duplicate flasks of medium. All of the soils decomposed some thiosulfate in 32 days, although the transformation was very meagre in soils 17, 18, 21, 23, and 28. These soils represent the most infertile ones of the group, some of which were quite acid in reaction. The most fertile soils, 19 and 20, decomposed the thiosulfate almost completely.

To a number of these cultures additions of sterile thiosulfate were made. The results after incubation for 11 additional days are given in the last two columns of table 3. In most cases decomposition continued. One culture each of soils 20 and 22 failed to develop, however; these cultures were the only ones that had decomposed the thiosulfate completely previous to the addition of more thiosulfate. It seems likely that in these instances the organisms had died as a result of exhaustion of the food material and the increase in acidity which generally accompanied extensive growth. After 10 days of further incubation, the thiosulfate had completely disappeared from all of the cultures except one each of soils 17, 20, 22, and 27.

In order to determine whether or not the initial reaction of the medium affected growth, media similar to medium III but adjusted over the range of pH 6.0 to 9.0 were prepared. Two of the neutral fertile soils, 20 and 25, were used. It is apparent from table 4 that the initial reaction exerted little effect on oxidation of thiosulfate. Organisms in soil 20 grew at all reactions and decomposed practically all of the thiosulfate at all reactions except pH 8.5. This exception appears to have no significance. With soil 25 decomposition was complete at both extremes of reaction. When further additions of thiosulfate were made to these solutions no growth occurred from cultures which had caused 100 per cent decomposition, but growth did develop from such cultures as from soil 20 of pH 8.0 and 9.0 which still contained some undecomposed thiosulfate at the time of the fresh addition. This suggests again that the bacteria tend to die out rapidly after the energy source is exhausted. In case the cultures are not transferred within a period of 2 or 3 weeks, the organisms

which cause abundant precipitation (probably *Th. thioparus*) are soon lost. Beijerinck noted that *Th. thioparus* died out in a short period on solid media (8).

Although soils seldom show the presence of organisms able to oxidize sulfur in acid media, they more frequently contain forms oxidizing sulfur to sulfate

TABLE 4

Decomposition of thiosulfate in media differing in initial reaction

SOIL NUMBER	pH OF MEDIUM	THIOSULFATE			
		12 days		28 days	
		Found	Decomposed	Found	Decomposed
		mgm.	*mgm.*	*mgm.*	*mgm.*
Control medium*	...	18.2	....	20.5	....
20	6.0	0	18.2	0	20.5
20	6.5	4.7	13.5	0	20.5
20	7.0	14.6	3.6	0	20.5
20	7.5	0	18.2	0	20.5
20	8.0	17.4	0.8	5.5	15.0
20	8.5	16.6	1.6	16.6	3.9
20	9.0	16.6	1.6	1.2	19.3
25	6.0	14.2	4.0	0	20.5
25	6.5	17.0	1.2	19.0	1.5
25	7.0	17.4	0.8	19.4	1.1
25	7.5	17.4	0.8	12.6	7.9
25	8.0	17.0	1.2	17.8	2.7
25	8.5	9.9	8.3	0	20.5
25	9.0	16.6	1.6	0	20.5

* Average of 7 flasks.

TABLE 5

Oxidation of elemental sulfur by crude cultures

SOIL SOURCE OF CULTURE	pH	TOTAL SULFATE-S IN 100 CC.
		mgm.
Control	9.0	14.9
20	5.8	80.3
20	5.6	118.9
25	5.8	32.3
25	5.2	84.9
26	6.0	31.7
26	6.0	52.9

in somewhat alkaline solutions. Crude cultures from three of the soils mentioned in table 3 were inoculated into an inorganic sulfur medium similar to medium I but containing 1 per cent $CaCO_3$. The results of table 5 indicate fairly active transformation of sulfur with appreciable increase in acidity.

Growth was accompanied by the development of a light turbidity of the medium. These cultures gave similar changes upon repeated transfers. No isolations of pure cultures were attempted with these solutions. It is reasonable to suppose that the growth was caused by organisms similar to *Th. thioparus*, since such cultures were subsequently isolated from these soils.

For the following studies medium IV was used. For a solid medium 1.5 per cent agar was added to this solution. Many of the cultures which effected appreciable oxidation of thiosulfate were used for the isolation of pure cultures. For this purpose nutrient and thiosulfate agar media were used.

The crude cultures showed a predominance of Gram-negative, small, rod-shaped or oval bacteria, some of which were motile. A great variety of protozoa (amoebae, flagellates, and ciliates) were encountered, particularly in the most active cultures. The bacteria were frequently found in the midst of precipitated sulfur, some of which was granular or crystalline but mostly in the amorphous state as highly refractive globules frequently having a diameter 10 or more times the longer dimension of the bacteria.

Only cultures from the New Jersey soils were used in the isolation studies. As a result of repeated plating, isolation, and culture on the solution medium a considerable number of pure cultures were obtained. Many of these failed to decompose thiosulfate and were discarded. The thiosulfate oxidizers were cultured on some of the customary laboratory media in order to determine how many of the cultures were distinct species. Morphological characteristics were also determined. More than 100 cultures were thus studied, and these yielded but three which could be considered as distinct, typical species.

Culture A

Thiosulfate solution. Uniformly turbid with no pellicle or material clinging to walls of the flask. Some whitish sediment, which is loose and unconsolidated, generally appears together with a thin incomplete membrane on the bottom of the flask. The reaction becomes acid within a few days, changing from an initial pH 7.8 to 5.8. This change is associated with the decomposition of a comparatively small quantity of thiosulfate. Further growth is probably prevented by the acidity, since the reaction seldom drops below pH 5.8. (See plate 2, fig. 11*c*.)

Sulfur solution medium of slightly alkaline reaction. No growth.

Nutrient agar slant. Soft, somewhat ropy growth, fairly abundant, raised, shining, and moderately spreading. Whitish in reflected light, brownish opalescence in transmitted light.

Nutrient agar stab. Fairly abundant mass of white mucoid material on the surface about the point of the stab. During the first few days not extending more than 1 cm. deep, gradually penetrating deeper, reaching the bottom of the tube. Growth white to cream colored confined close to the line of inoculation.

Nutrient agar plate. Growth slow. In 4 to 5 days surface colonies up to

1 mm. in diameter, round, raised, moist, and colorless, similar to drops of thin starch paste. Deep colonies tiny, lens shaped up to about 0.5 mm. in longer dimension in 5 days. (See plate 2, fig. 9.)

Thiosulfate agar slant. Very thin and practically colorless, not penetrating below the agar surface. In most cases accompanied by some sulfur which is precipitated as a white frosty film on the surface of the slant.

Thiosulfate agar stab. No appreciable growth on the surface. Very thin growth and shadowy in outline extending slightly from the line of the stab, like a thin veil.

Thiosulfate agar plate. Colonies develop slowly, becoming white from sulfur which is precipitated. Sub-colonies are lens shaped or round from pin-point size up to 1 mm. in long dimension in 1 or 2 weeks; surface colonies small, round, and moist reaching 1 to 1.5 mm. in diameter. Crystals of calcium sulfate appear throughout the agar as growth progresses. (See plate 1, fig. 4a.)

Nutrient solution. Slight turbidity. Tendency for formation of pellicle, which becomes complete and gelatinous. Most characteristic is the formation of a long streamer-like network extending from the surface to the bottom of the tube and forming some sediment.

Potato slant. Limited development of cream colored growth, moist, shining, and slightly brown.

Litmus milk. Slow development of slight alkalinity.

Gelatin stab. Mucoid growth at point of inoculation or slightly spreading in a circular zone at the surface. Very thin growth below the surface. No liquefaction during first week. Slight saccate liquefaction in a few weeks.

Morphology. Small, Gram-negative, non-sporulating, non-motile, short rods or coccoid cells varying in size from 0.4–0.8 x 0.6–1.8 μ. Average size 0.6 x 1.2 μ. The organism takes the common stains readily. (See plate 1, fig. 4b.)

Source. This organism was recovered from soils 20 and 25. The cultures used for physiological studies came from soil 20.

Culture B

Thiosulfate solution. Comparatively slight evidence of growth. Occasionally light turbidity during first few days. No pellicle. Generally the small amount of precipitate common to the medium becomes drawn together in flocculent aggregates. Occasionally a small amount of elemental sulfur appears as a result of secondary reactions. Slow decomposition of thiosulfate is accompanied by an increase in alkalinity, which may change from pH 7.8 initially to over pH 9.0.

Sulfur solution medium. No growth.

Nutrient agar slant. Smooth, thin, spreading, fairly abundant growth, soft and buttery in consistency. White to gray in reflected light, light brown in transmitted light. Young cultures slightly fluorescent.

Nutrient agar stab. Smooth, spreading growth on surface with dense center and serrated edges. Develops well into the medium as granular growth confined closely to the line of the stab.

Nutrient agar plate. Colonies circular, fairly large on surface (5 mm. in diameter in 2 days). Flat with raised center, surface granular. Deep colonies, tiny lens shaped (about 1 mm. in long dimension). Young cultures fluorescent. (See plate 2, fig. 7.)

Thiosulfate agar slant. Thin growth spreading over surface as tiny droplets, some cases scarcely visible, in others fairly heavy and white as a result of appearance of elemental sulfur. Growth penetrates the agar and produces a fine granular opacity beneath the surface of the slant, particularly pronounced at the base.

Thiosulfate agar stab. Little or no growth on surface, except occasional droplets. Grows well to about 1 cm. deep with granular particles spreading from the line of stab. Growth below this region thin and limited.

Thiosulfate agar plate. Colonies thin and diffuse, somewhat denser about the center. Become as large as 5 mm. in diameter in 3 days. Tendency to grow into the agar. When reaching the surface, the center of the colony appears as a tiny moist drop. (See plate 1, fig. 1a.)

Nutrient solution. Thin fragile pellicle which settles to bottom. Dense uniform turbidity. Abundant sediment.

Potato slant. Fairly abundant, somewhat spreading mucoid, cream colored growth turning brown.

Litmus milk. Turns strongly alkaline with no digestion.

Gelatin stab. No liquefaction in 4 weeks. Uniform gray growth over surface. Scant development along the line of stab.

Morphology. Small coccoid, Gram-negative, non-sporulating cells frequently occurring in pairs appearing like rods with terminal granules. Actively motile. Vary in size from 0.3–0.7 x 0.4–1.2 μ. Average size 0.5 x 0.7 μ. Readily stained. (See plate 1, fig. 1b.)

Source. Recovered from soils 20, 25, and 26, cultures from the first two sources were used for physiological studies.

Culture C

Thiosulfate solution. Growth is very rapid, becoming turbid in 24 hours as a result of initial precipitation of sulfur. As growth proceeds the sulfur either accumulates as a complete membrane mixed with bacterial cells on the surface or is deposited on the walls and bottom of the flask. The medium remains somewhat turbid. Complete decomposition of the thiosulfate occurs within a week or 10 days, and the reaction becomes acid, frequently dropping from the initial pH 7.8 to as low as pH 4.0–4.5. Cultures must be frequently transferred (every 2 or 3 weeks). (See plate 2, fig. 11*b*.)

Sulfur solution medium. Characteristics uncertain. Oxidizes the sulfur, if at all, much more slowly than thiosulfate. Forms some turbidity. The reaction becomes acid.

Thiosulfate agar slant. Thin, veil-like droplet film developing on the surface. The small masses of cells become brown in old cultures. Throughout the agar, tiny colonies develop, which together with small aggregates of pre-

cipitated sulfur and crystals of various sulfates give a uniform milky appearance to the entire agar contents. The organism dies out upon such media if not frequently transferred (every 1 to 3 weeks). Growth is much the same as for *Th. thiooxidans* on thiosulfate agar.

Thiosulfate agar stab. On the surface, a thin film of droplets develops about the point of inoculation. These droplets become coated with sulfur. Upon aging they turn brown. Growth follows the stab to a depth or 2 or 3 cm. forming a thin veil showing some precipitation of sulfur.

Thiosulfate agar plate. Colonies develop slowly. When heavily inoculated, only microscopic colonies appear, which cause the entire agar plate to become cloudy with precipitated sulfur interspersed with crystals of calcium sulfate with clear zones about the colonies. When few or scattered, the colonies are larger, moist, and soft. Surface colonies are circular from 1 to 2 mm. in diameter in a period of several days to one month; sub-colonies are lens shaped up to 1 mm. in long dimension (plate 1, fig. 5*a*). Colonies first appear white with precipitated sulfur and later become brown but are still impregnated with sulfur, which appears microscopically as variously sized, highly refractive, yellow globules, most of which vary in size between 1 and 10 μ in diameter. (See plate 1, fig. 6.)

No growth on organic media.

Morphology. Tiny coccoid to oval, Gram-negative, non-sporulating cells. Pure cultures non-motile. Frequently encountered mixed with organism B, which is not readily distinguishable morphologically except by its active motility. Varies in size from 0.3–0.7 x 0.4–0.9 μ. Average size 0.5 x 0.7 μ. Readily stained. (See plate 1, fig. 5*b*.)

Source. Recovered from soils 20, 22, 25. Culture used for physiological studies obtained from soil 25.

Culture C is hardly distinguishable morphologically from *Th. thiooxidans*, which measures 0.4–0.6 x 0.5–0.9 μ with average of 0.5 x 0.8 μ. *Th. thiooxidans* is, however, actively motile by means of a single polar flagellum which has a ribbon-like appearance and may be several times the length of the cell. (See plate 2, fig. 10.) It was stated in earlier reports that *Th. thiooxidans* is also Gram-positive (69). More recent repeated tests have shown that the organism is Gram-negative. It cannot be distinguished from other members of the genus *Thiobacillus* by the Gram reaction [see in this connection (15, 16, 49)].

Culture C appears to be a strict autotroph and in this and other respects shows physiological characteristics practically identical with *Th. thioparus* and may be considered as this species, at least tentatively. *Th. thioparus* was reported by Beijerinck (8) as being actively motile and having somewhat larger dimensions (3 x 0.5 μ).

Culture A is a facultative autotroph which does not appear to have been described previously. By reason of its physiological behavior it belongs to the genus *Thiobacillus*. The name *Thiobacillus novellus* nov. sp. is herewith given to this organism.

Culture B is very similar to the cultures of Trautwein in its activity upon the thiosulfate medium and development upon the organic media (62, 63). Trautwein's cultures were named *Th. trautweinii* by Bergey (9). In view of the fact that it was found in studies to be reported subsequently that neither this organism nor culture B are autotrophic, their inclusion in the genus *Thiobacillus* is unwarranted. Culture B is somewhat smaller in size than *Th. trautweinii*. Trautwein gave the average dimensions of his organisms as 0.4 x 1.3 μ. Measurements of his cultures made in this laboratory average 0.6 x 1.6 μ. Trautwein's cultures T and K have been preserved here for about 10 years and have been used in various comparative tests with the three cultures already mentioned. These organisms have morphological and colony characteristics very similar to those of culture B (plate 1, fig. 2a, 2b, 3a, 3b; plate 2, fig. 8). A third culture of Trautwein (W) was lost from our collection of cultures.

The characteristics of the various organisms when developing upon the thiosulfate medium are compared in table 6.

TABLE 6

Development of organisms in thiosulfate solution

CULTURE	AGE	pH*	TITRATION OF 5 CC. WITH 0.01 *N* IODINE	GROWTH CHARACTERISTICS
	days			
Control	14	7.8	20.5	—
B	14	8.8	15.9	Slight flocculent sediment
Trautwein (T)	14	8.2	16.3	Slight flocculent sediment
Trautwein (K)	14	8.8	15.0	Slight flocculent sediment
A (*Th. novellus*)	14	5.6	17.5	Turbid. Slight bottom film
C (*Th. thioparus*)	14	4.6	0	Heavy precipitation of sulfur
Th. thiooxidans	14	1.4	0	Turbid

* The initial reaction for *Th. thiooxidans* was pH 6.0, since it fails to develop in solution media at alkaline reactions.

It seems evident that the transformation of thiosulfate by these organisms must involve a variety of reactions, since both the visual characteristics of growth and the changes in pH are distinctly different for the various species. A discussion of these differences and of the influence of environmental conditions on the course of the processes is reserved for subsequent reports.

SUMMARY AND CONCLUSIONS

Only 2 of the 29 soils examined indicated the presence of autotrophic bacteria oxidizing sulfur under acid conditions, and 1 of these soils had previously been inoculated with *Th. thiooxidans* and had received an application of sulfur. Organisms able to effect the oxidation of sulfur in somewhat alkaline media are more readily detected. In all of the 28 soils, organisms were found which decomposed thiosulfate in mineral solution. The rapidity and extent of oxidation varied with the soil. Decomposition of thiosulfate by crude cultures took place in mineral media over a wide range of reaction (pH 6.0–9.0).

Three cultures which were physiologically distinct from one another were obtained from the soils:

1. One of the cultures (A), which is a facultative autotroph, seems to be a new species and is designated by the name *Thiobacillus novellus* nov. sp. It oxidizes thiosulfate with acid formation and fails to oxidize elemental sulfur. It is a small, Gram-negative, non-motile, non-sporulating rod 0.6 x 1.2μ in size. It grows well on both organic media and mineral media containing thiosulfate.

2. Culture B is closely related to cultures of Trautwein named *Th. trautweinii*, Bergey et al. In the oxidation of thiosulfate by these bacteria the pH of the media increases. They make little visible evidence of growth in mineral thiosulfate solutions and grow on a variety of organic media. These cultures are not autotrophic and do not belong to the genus *Thiobacillus*.

3. Culture C is identical with or at least closely related to *Th. thioparus*, Beij. During oxidation of thiosulfate, the medium becomes acid and elemental sulfur is precipitated. This is a strict autotrophic bacterium, a small, non-sporulating, Gram-negative, non-motile rod 0.5 x 0.7μ in size.

Some of the cultural and morphological characteristics of these organisms are presented and compared with *Th. thiooxidans*, Waks. and Joffe, which is also a Gram-negative tiny rod 0.5 x 0.8 μ in size.

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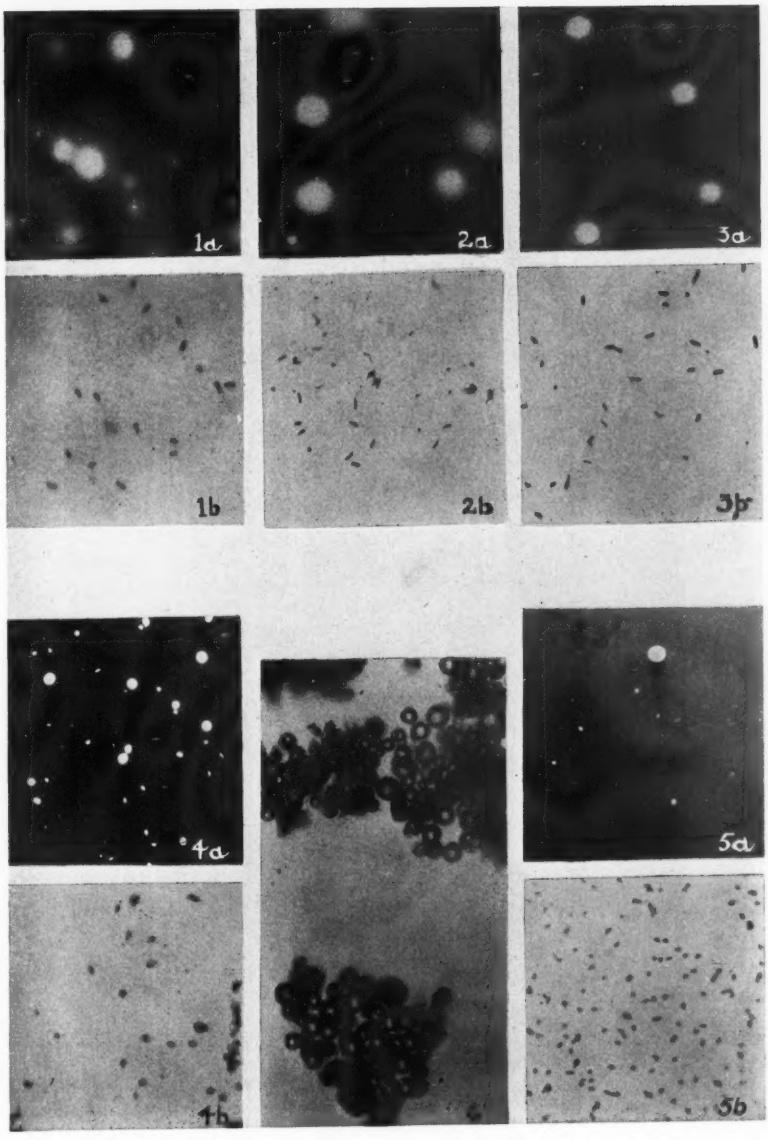
PLATE 1

COLONY DEVELOPMENT DURING 12 DAYS ON THIOSULFATE AGAR. 1.8 ×

FIG. 1a. Culture B.
FIG. 2a. Culture T (*Th. trautweinii*).
FIG. 3a. Culture K (*Th. trautweinii*).
FIG. 4a. Culture A (*Th. novellus*).
FIG. 5a. Culture C (*Th. thioparus*).

PHOTOMICROGRAPHS OF CELLS FROM THIOSULFATE AGAR CULTURES, 5 DAYS OLD. 1000 ×

FIG. 1b. Culture B.
FIG. 2b. Culture T (*Th. trautweinii*).
FIG. 3b. Culture K (*Th. trautweinii*).
FIG. 4b. Culture A (*Th. novellus*).
FIG. 5b. Culture C (*Th. thioparus*).
FIG. 6. Globules of sulfur from a colony of culture C developing upon thiosulfate agar. 468 ×.



UNIV. OF MICH.

PLATE 2

COLONY DEVELOPMENT DURING 5 DAYS ON NUTRIENT AGAR. 1.8 ×

FIG. 7. Culture B.

FIG. 8. Culture T (*Th. trautweinii*).

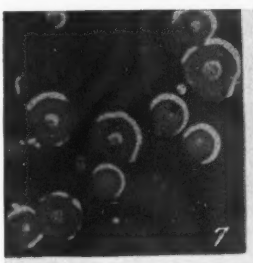
FIG. 9. Culture A (*Th. novellus*).

FIG. 10. Photomicrograph of *Th. thiooxidans* showing flagellation. 2160 ×.

FIG. 11. *a*. Uninoculated thiosulfate solution medium.

b. Thiosulfate solution medium supporting growth of culture C (*Th. thioparus*). General turbidity with sulfur precipitation in culture 7 days old.

c. Thiosulfate solution medium supporting growth of culture A (*Th. novellus*). Uniformly turbid solution of culture 7 days old.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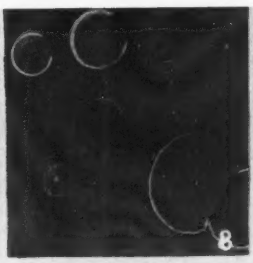
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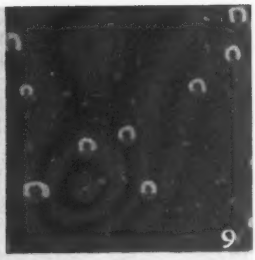
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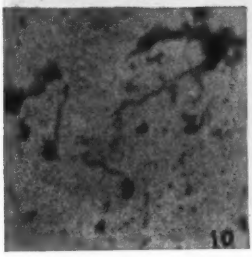
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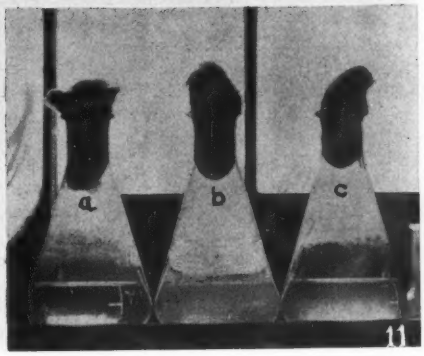
a
b
c
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THE NOMENCLATURE OF THE COWPEA GROUP OF ROOT-NODULE BACTERIA[1]

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From the time the root-nodule bacteria of leguminous plants were first studied there has been considerable interest in the differentiation of species. At first it was believed that a single species of bacteria was responsible for the production of nodules on all legume plants. As the study of these organisms continued, however, it was learned that the organisms responsible for the production of nodules on some legumes are different in certain morphological or physiological characteristics from those producing nodules on other legumes. Hence, there has arisen the problem of the differentiation of species.

In a number of publications it has been suggested that the organisms producing nodules on certain plants be recognized as constituting separate species. These reports have been adequately reviewed by Buchanan (2) and by Baldwin and Fred (1), the latter investigators having made, in addition, the definite suggestion that the organisms of certain cross-inoculation groups be considered as constituting separate species.

Because of a lack of information concerning the bacteria of certain legumes or groups of legumes, the classification and nomenclature of the root-nodule bacteria have not yet been completed. Perhaps the outstanding example of this is in the case of the organisms producing nodules on cowpea and other plants of the same cross-inoculation group.

There has been some uncertainty as to whether or not the cowpea root-nodule bacteria should be classified with the soybean bacteria. It was early recognized by Kirchner (5) that the soybean bacteria are different from most of the other root-nodule organisms. He suggested that the soybean organisms be placed in a separate species, for which he proposed the specific name *japonicum*, which is now recognized as valid and is in rather general use by American bacteriologists. Numerous experiences of a practical nature have shown the desirability of inoculating soybean seed with organisms isolated directly from soybean root-nodules. Furthermore, numerous controlled cross-inoculation experiments have indicated that the soybean bacteria infect only soybean plants and that root-nodule organisms from other plants do not infect soybeans. As a result the belief that the soybean bacteria are distinctly different from the other root-nodule organisms, including those of cowpeas, has become rather general.

What may seem as an apparent contradiction to the generally accepted belief concerning the failure of soybean and cowpea organisms to cross-inoculate are the results reported in recent years by Leonard (6), Sears and Carroll (7), Hansen and Tanner (4), and Carroll (3). These investigators found that certain strains of cowpea bacteria are capable of producing nodules on soybeans and that certain strains of soybean bacteria are capable of producing nodules on cowpeas. The interchangeability has not been considered perfect in all cases, however, as in certain cases where soybean organisms were found to produce nodules on cowpeas, it was observed that the nodules were not typical of those produced on cowpeas by cowpea organisms. Furthermore, not all strains of the cowpea organisms nor of the soybean organisms tested were found to cross-inoculate.

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[1] Journal Paper No. J 165 of the Iowa Agricultural Experiment Station, Project No. 226.

This situation has led to a little uncertainty as to the proper classification of the organisms of these two cross-inoculation groups. Inasmuch as the principal criterion for species differentiation within the genus Rhizobium has been the ability to inoculate plants, it has not been known definitely whether the organisms of the soybean and cowpea root-nodules should be considered as belonging to the same or to different cross-inoculation groups.

In order to obtain additional information on this problem, the following cross-inoculation experiments with a number of strains of soybean and cowpea root-nodule bacteria were conducted.

EXPERIMENTAL

Five strains of organisms that were at one time isolated from cowpea root-nodules and fifteen strains similarly isolated from soybean root-nodules were tested for their ability to cross-inoculate. In the first experiment the five

TABLE 1

The formation of nodules on cowpea and soybean roots by five strains of cowpea root-nodule bacteria

STRAINS OF COWPEA BACTERIA	NODULATION OF COWPEA PLANTS*		NODULATION OF SOYBEAN PLANTS	
	First	Second	First	Second
603	+	+	−	−
605	+	+	−	−
607	+	+	−	−
608	+	+	−	−
609	+	+	−	−
Control	−	−	−	−
Control	−	−	−	−

* + indicates that nodules were produced; − indicates that no nodules were produced.

strains of cowpea organisms were used to inoculate both soybean and cowpea seeds. In the second experiment the fifteen strains of soybean bacteria were similarly used to inoculate both kinds of seed.

The seeds were first sterilized with hydrogen peroxide and then inoculated with the various strains of bacteria. After being inoculated the seeds were planted in sterile sand, where they were permitted to grow in the greenhouse for 3 weeks. At that time the plants were removed from the sand cultures and observed for nodulation of the roots. The results of the two tests are reported in tables 1 and 2.

Quadruplicate controls of uninoculated seeds were carried along in each test, and in no instance was there any sign of nodule development on any of the control plants. Furthermore, there was good agreement of duplicate cultures of the inoculated plants, and in no case did there appear to be any serious difficulty in determining whether or not nodules had been produced.

The data show rather definitely that, whereas all the cowpea bacteria produced nodules on cowpea roots, none of them produced nodules on soybean roots. On the other hand, whereas all of the soybean bacteria produced nodules on soybean plants, eight of them also produced nodules on cowpea plants. Three of the soybean cultures definitely failed to produce nodules on cowpeas, and four others each failed to produce nodules in one of the duplicate tests, but produced nodules in the other test in each case.

TABLE 2

The formation of nodules on cowpea and soybean roots by fifteen strains of soybean root-nodule bacteria

STRAINS OF SOYBEAN BACTERIA	NODULATION OF COWPEA PLANTS*		NODULATION OF SOYBEAN PLANTS	
	First	Second	First	Second
401	+	−	+	+
402	+	+	+	+
403	+	−	+	+
404	−	−	+	+
405	−	−	+	+
406	+	+	+	+
407	+	+	+	+
408	−	−	+	+
410	+	+	+	+
411	+	−	+	+
412	+	−	+	+
413	+	+	+	+
414	+	+	+	+
415	+	+	+	+
416	+	+	+	+
Control	−	−	−	−
Control	−	−	−	−

* + indicates that nodules were produced; − indicates that no nodules were produced.

DISCUSSION

The results, in general, are in accord with those reported by Leonard (6), Sears and Carroll (7), Hansen and Tanner (4), and Carroll (3), who obtained cross-inoculation with certain strains of the cowpea and soybean bacteria, but not with other strains. Results like those obtained in the test with the cowpea bacteria lead one to believe that the organisms that infect these two legumes are distinctly different. But the results of the tests with the soybean bacteria indicate that there is at least some relation between the two organisms and their abilities to infect plants. This naturally leads to the question of the species relationship of the two organisms.

In view of the results obtained by the several investigators of this problem, it seems logical that the bacteria producing nodules on the roots of soybeans

and cowpeas should be considered as belonging to the same species, instead of constituting separate species as they have been considered rather generally in the past. Carroll was undoubtedly of this opinion when he grouped the soybean bacteria in the cowpea cross-inoculation group. Further, it is assumed that Baldwin and Fred (1) were of somewhat the same opinion when they failed to give the organisms of the cowpea group a specific designation in their paper on the nomenclature of the root-nodule bacteria. If the cowpea and soybean organisms are to be considered as belonging to the same species, then according to the rule of priority in botanical nomenclature, they would be designated by the specific name proposed for the soybean organism by Kirchner in 1895 and now in general use in bacteriological literature. Thus the generic and specific name for this group would be *Rhizobium japonicum*.

The observed irregularities in the interchangeability of organisms to the various hosts within the cross-inoculation group may well be interpreted as being due to biologic variation of the strains of bacteria within the species. Variability in various physiological and serological characters among strains within species of Rhizobium has been quite definitely shown, and it is just as reasonable to assume that there may be a similar variability in the physiology of the organisms with respect to the interchangeability of hosts within a cross-inoculation group. As Carroll has pointed out, it is quite reasonable to assume that there may be some variability or irregularities in the infection of plants in a group of legumes as large and as variable as that of the cowpea group, now consisting of 17 known genera and 33 species of legumes. On the other hand, where the cross-inoculation groups are made up of a small number of closely related legumes such as in the alfalfa and clover groups, it is only natural that a greater uniformity of inoculation ability would be observed.

CONCLUSIONS

In view of the fact that certain strains of soybean bacteria have been found capable of producing nodules on cowpea roots, and that certain strains of cowpea bacteria have been found capable, by other investigators, of producing nodules on soybean roots; it is suggested that the root-nodule bacteria of soybeans and cowpeas be brought together in the one species now generally designated *Rhizobium japonicum* (Kirchner) Baldwin and Fred.

Different strains of this species of Rhizobium exhibiting difference in their abilities to produce nodules on certain plants within the cross-inoculation group may well be considered as biological variations within the species.

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A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WINDBLOWN SOIL: IV. SHACKLEFORD BANK, NORTH CAROLINA

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An article by MacCarthy on the "Beach sands of the Atlantic Coast" (2) suggested to the writer that the high $CaCO_3$ content of the sand in the southern part of this coast might yield an interesting bacterial flora; consequently, on March 30, 1931, sand was collected from Shackleford Bank, N. C. for this study.[1]

METHODS AND MATERIALS

The methods and materials used were in general the same as those previously described (5, 6, 7). The sand was collected at three levels, 6, 12, and 24 inches, from the outer face of the Bank, about 100 feet from the tide (approximately half tide) and about 5 feet from the base of the slope. It was brought to Wellesley as rapidly as possible by motor and train and plated in the same manner as in previous work. The indicator media varied somewhat from those used in the previous studies. When the reaction in glucose gelatin was uncertain it was checked in glucose broth or agar. The latter in some cases was made with yeast extract instead of the usual beef-peptone base. Glucose positives were inoculated into sucrose broth or agar, the latter also made with yeast extract. Litmus lactose agar was used for the lactose fermentation test. Nitrate broth was used as formerly.

The presentation of results has been delayed because of the hope that another study of a similar soil might be made, but as this seems impracticable at present the following brief report is presented.

RESULTS

The average annual precipitation for the 5 years previous to the collection was 54.83 inches, with a variation of 40.70–73.26 inches, and the average annual mean temperature for this period was 63.96°F. with a variation of 13°F.–96°F. (8).

Analysis of soil

The high CO_3 content noted by MacCarthy (3) for a point $\frac{1}{4}$ mile northwest of Fort Macon, Beaufort, N. C., which is directly across the inlet from the

[1] The collection was made possible by the courtesy of Dr. S. Hildebrand of the U. S. Bureau of Fisheries Laboratory, at Beaufort, N. C., to whom I wish to express my thanks.

place at which the present collection was made, is "7.75 percentage loss by leaching." This was obtained with HCl, whereas the present data were obtained from water-soluble salts only.

TABLE 1

Physical characters of soil

DEPTH	SIZES OF SOIL PARTICLES				WATER RELATIONS				COMBUSTIBLE MATERIAL
	More than 1 mm.	1 to 0.5 mm.	0.5 to 0.1 mm.	Less than 0.1 mm.	Water content	Water capacity	Optimum* water content	Relative† water content	
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	3.3	18.7	45.2	32.9	2.01	21.00	14.70	9.57	0.80
12	0.2	12.3	62.2	25.0	1.04	23.00	16.10	4.52	0.30
24	0.6	5.0	45.9	48.4	3.89	25.00	17.50	15.56	0.25
All	1.4	12.0	51.1	35.4	2.31	23.00	16.10	10.04	0.45

* 70 per cent of capacity.

† Percentage of capacity present.

TABLE 2

*Chemical character of soil**

DEPTH	REACTION	TOTAL SOLUBLE SALTS	CO_3	SO_4	Cl	NO_3, etc.
inches	*pH*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	9.0	0.00918	0.00579	0.000834	0.00054±	0.00188
12	9.2	0.00992	0.00626	0.001803	0.00058±	0.00143
24	9.2	0.01294	0.00549	0.002188	0.00057±	0.00513
All	9.0–9.2	0.01068	0.00585	0.001608	0.00056±	0.00281

* The chemical analysis, except for the pH values, was made by Miss Miriam Dice, who used the same methods as she (5, 6) used in two of the previous analyses.

TABLE 3

Average numbers of organisms present in a gram of fresh soil

DEPTH	NUMBER OF PLATES COUNTED	TOTAL COUNT	BACTERIA OR YEAST	ACTINOMYCETES	FUNGI
inches			*per cent*	*per cent*	*per cent*
6	12	26,084	99.69	0.00	0.31
12	12	18,750	94.70	4.44	0.85
24	12	134,166	99.62	0.18	0.18
All	36	59,666	99.12	0.61	0.27

The high actual and relative water content of the 24-inch layer as compared with the percentages for the 12-inch layer possibly may account for the difference in the numbers and proportions of the organisms in the two. Cobb (1) found water to be the limiting factor in forest soils and cites numerous references for (and also against) this relationship.

One tube (culture 24–20) gave a basic reaction in litmus lactose agar but contained gas bubbles. Three repetitions of the test gave the same result, after which the culture was lost and no further work could be done. No gas appeared in glucose or sucrose and no other culture produced gas in any sugar. According to Merrill (4), only members of the genus Mycobacterium are able completely to ferment sugars to CO_2 and H_2O without the formation of organic acids. Culture 24–20 appeared to do this in lactose only. One difficulty, however, lies in the fact that Mycobacteria are strictly aerobic and unable to utilize lactose, whereas this culture showed bubbles of gas throughout the lactose agar.

TABLE 4

Colors of colonies on representative plates and cultures picked for study

GROUP	TOTAL NUMBER	WHITE	YELLOW	ORANGE	PINK	FLUORESCENT
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Colonies	1,210	83.17	8.22	0.49	0.70	7.42
Cultures	119	51.30	22.70	21.00	0.80	4.20

TABLE 5

Morphology and physiology of the organisms studied

DEPTH	TOTAL NUMBER	COCCUS OR COCCOID	PLEOMORPHIC	RODS		GRAM'S*		FERMENTERS			DIGESTORS		NO_3 REDUCERS†	
				Non-spore	Spore	Positive	Negative	Glucose	Sucrose	Lactose	Gelatin	Casein	Positive	Negative
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	40	10.0	35.0	55.0	0.0	37.5	55.0	27.5	20.0	0.0	45.0	26.3‡	45.0	37.5
12	39	51.3	30.8	28.2	2.6	15.4	74.4	17.9	30.8	2.6	69.2	28.2	41.2	56.4
24	40	32.5	25.0	42.5	0.0	17.5	72.5	20.0	12.5	2.5	45.0	25.0	47.5	50.0
All	119	31.1	26.9	41.2	0.8	23.6	67.2	26.5	16.8	1.7	52.9	26.5	44.6	47.9

* Others were variable.

† The remaining percentage did not grow.

‡ Two cultures died before the test.

In regard to the single spore-former found the writer does not wish to imply that this, or a similar soil, is almost wholly lacking in spore-formers. Another analysis might yield a different result. The fact remains, however, that of 119 cultures picked from plates in an effort to get as many kinds of colonies as possible for study, only one form showed spores when cultured and stained according to the usual technique. In the previous studies, made as far as the author is aware in exactly the same way, 42.60 per cent (5), 22.47 per cent, and 19.67 per cent (6) of the cultures developed spores. An effort was made to check the heat resistance by exposing 25 of the 6-inch cultures to 80°C. for 10

minutes. After 2 days' incubation at 28°C. those that appeared cloudy were transferred to broth. Twenty of these were definitely negative, but five showed by growth that they had resisted the heat. Four of the five were pleomorphic forms, one gram-negative, and three gram-positive. This would suggest Mycobacterium, with one gram stain an error. The fifth form was a gram-negative short rod, probably a thermophile. It would be very desirable to do further work along this line.

Culture groups

The most conspicuous and homogeneous group was composed of 23 pale orange, or cream-colored cultures, oval-pleomorphic in form, gram-positive, non-fermenting, non-digesting, but strongly reducing organisms. A second fairly homogeneous group was composed of 36 pale yellow, white, or fluorescent cocci, or very short rods. These were gram-negative liquefiers which did not ferment sugars or, usually, reduce nitrates. The remaining 60 forms arranged themselves in small groups with various combinations of cultural characters.

Comparison of the north and south Atlantic soils

Although the soil of the north Atlantic coast was much coarser in quality than that of the south Atlantic coast, the water relations in the two were very similar. The southern soil was much more alkaline and contained larger amounts of soluble salts, but the water-soluble carbonate content was not very large. As water-soluble analysis had been used in previous studies, acid treatment for total carbonates would not have yielded comparable results.

A lower total number of organisms in the southern soil was composed almost wholly of bacteria, or yeasts, whereas the percentages of actinomycetes and fungi were much higher in the northern soil. When the previous discussion on the Arizona soil (5) regarding percentages of actinomycetes in soils is recalled, the distribution of these organisms in the soils in question is not very enlightening. Aridity cannot be the prime factor, as the water relations in the two soils were very similar. Nor can alkalinity account for the larger number in the northern region, since the southern soil had a higher pH.

The percentages of colored colonies found on plates were not widely different in the two soils, the larger percentage of fluorescent forms in the northern soil being the most outstanding difference. The cultures from the southern soil had the larger percentage of yellow and orange and the smaller percentage of pink forms. The northern soil cultures had a small percentage of brown and no fluorescent forms, but the reverse was true of the southern cultures.

Morphologically and culturally the forms studied were rather similar, the greatest variation noted being the almost total absence of spore-formers in the southern soil, as against 19.67 per cent in the northern soil. Casein digestion and nitrate reduction were more pronounced in the southern soil.

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A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WINDBLOWN SOIL: V. MONTEREY PENINSULA, CALIFORNIA

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This paper is the fifth in a series of brief surveys made in the attempt to compare bacteriologically similar soils under various climatic conditions (1). The work was done at the Hopkins Marine Station of Stanford University, at Pacific Grove, California.[1]

METHODS AND MATERIALS

The methods were in general those used in the previous work. Because the dunes all along the coast of the Peninsula are constantly traversed by people and animals it was impossible to find a region not influenced by human contact. The best that could be done was to select places for collection as far as possible from visible tracks. The first collection was made on July 8, 1932 from the smooth south-by-west face of a low dune south of Asilomar, and approximately 100 yards from the ocean. Collections were made from three levels, as formerly; namely, 6, 12, and 24 inches. On October 10, after 3 months without rain, a second collection was made approximately 1 mile farther south. The 6-inch layer in this case was very dry, and the level could not be accurately maintained. An analysis showed that this upper section contained 0.12 per cent water; the 12-inch layer, 1.14 per cent; and the 24-inch layer, 2.08 per cent. No other chemical or physical characters were studied for this collection. The sand in both cases was brought immediately to the laboratory and plated as usual in nutrose agar. For isolation of pure cultures yeast-autolysate agar, having the following composition was used: K_2HPO_4, 1 gm.; $MgSO_4$, 5 gm.; agar, 20 gm.; tap water, 900 cc.; yeast autolysate, 100 cc. From the plates of both collections colonies were fished for further study. Morphological and physiological studies were made. Glucose gelatin, sucrose, lactose, nitrate, and nutrose agar were used for the tests.

CLIMATE

The climatological data from Del Monte (2) are used for 1929, 1930, and 1931. As there were no reports for that station for 1927 and 1928 the figures for Salinas are substituted, since the data from that station agreed very closely

[1] With the cordial coöperation of various members of the staff, to whom the writer wishes to express her sincere thanks.

with those for Del Monte in the other years. The average annual rainfall for the 5 years previous to the study was 11.69 inches, with a variation of 7.21–16.06 inches. The average annual mean temperature for this period was 55.6°F. with a variation of 24°F.–95°F.

TABLE 1

Physical character of the soil

DEPTH	SIZES OF SOIL PARTICLES				WATER RELATIONS				COMBUSTIBLE MATERIAL
	More than 0.589 mm.	0.588–0.295 mm.	0.295–0.104 mm.	Less than 0.104 mm.	Water content	Water capacity	Optimum water content*	Relative water content†	
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	0.718	65.463	33.636	0.099	2.28	24.55	17.19	9.40	0.03
12	0.553	60.182	39.126	0.052	2.89	23.15	16.21	12.48	0.11
24	0.190	54.631	45.188	0.030	2.94	23.37	16.36	12.58	0.09
All	0.493	60.269	39.138	0.058	2.70	23.69	16.58	11.49	0.07

* 70 per cent capacity.

† Percentage of capacity.

TABLE 2

Chemical character of the soil

DEPTH	REACTION	WATER-SOLUBLE SALTS*				
		Total	CO_3	SO_4	Cl	NO_3, etc.
inches	*pH*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
All	5.9–6.1	0.00452	0.00142	0.00052	0.00075	0.00183

* Analysis by Miss Miriam Dice, who had made three of the other analyses.

TABLE 3

Organisms per gram of fresh soil

DEPTH	NUMBER OF PLATES		TOTAL COUNT		YEASTS OR BACTERIA		ACTINOMYCETES		FUNGI	
	July	October	July	October	July	October	July	October	July	October
inches					*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	10	3	41,640	10,000	48.99	70.00	48.75	30.00	3.26	0.00
12	8	3	135,875	126,700	59.81	57.39	38.73	41.28	1.38	1.34
24	8	3	10,162	37,700	65.06	17.77	33.57	80.37	1.35	1.85
All	26	9	62,559	58,133	57.75	49.54	40.67	49.08	1.57	1.37

ANALYSIS OF SOIL

The sand was passed through a Tyler Standard Screen Scale, of which the sieves with openings as near as possible those previously used were selected. It will be seen from table 1 that the sand was exceedingly uniform in size, over 99 per cent of it being between 0.1 and 0.6 mm. in diameter.

All depths considered, 0.49 per cent of the soil was larger than 0.6 mm., and 99.47 per cent, smaller. If the water content of the second area is assumed to have been approximately the same in July as that of the first area, the soil lost in 3 months without rain 90.79 per cent, 60.55 per cent, and 29.25 per cent of dry weight respectively in the three depths.

The highest number of organisms at 12 inches, the lowest at 6 inches, with 24 inches intermediate agrees with the results for Arizona and Indiana (1, I, III), but only in the case of Arizona do numbers and water content correlate. Massachusetts dunes (I, II) had the highest number at 6 inches and decreased in count downwards, whereas the water content was reversed.

There was gas in glucose and sucrose for one form. This and the large percentage of lactose fermenters are rather unusual, and might indicate contamination by animals except for the fact that there was no gas in lactose. Morphologically the lactose fermenters were divided as follows: 6 inches, 7 short

TABLE 4

Colors of colonies on representative plates and of cultures selected for study

GROUP	TOTAL NUMBER	WHITE	YELLOW	ORANGE	RED	FLUORESCENT	BROWN
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plates*	3,850	99.14	0.54	0.10	0.00†	0.21	0.00†
Cultures	92	52.17	30.43	5.43	9.78	0.00	2.17

* No colors observed on 24-inch plates.

† No red or brown colonies evident on plates, but cultures developed colors.

TABLE 5

Morphology and physiology of forms selected for study

DEPTH	NUMBER STUDIED	MORPHOLOGY OF FORMS						PHYSIOLOGICAL REACTIONS					
				Rods				Fermenters			Digesters		
		Coccus	Pleomorphic	Non-spore	Spore	Yeasts	Gram-positive*	Glucose	Sucrose	Lactose	Gelatin	Casein	NO_3 reducers
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	31	19.35	0.00	70.98	9.67	0.00	67.74†	58.06	45.16	29.03	35.48	29.03	16.12
12	29	6.89	6.89	82.77	3.44	0.00	37.93‡	41.37	34.48	13.79	48.27	34.48	10.34
24	32	9.37	12.50	43.70	28.12	6.25‖	62.50§	56.25**	37.50**	31.25	28.12	37.50	34.37
All	92	11.96	6.52	65.21	14.12	2.17	56.52	52.17	39.13	25.00	36.95	33.69	20.65

* All others were negative.

† All cocci gram-positive and 60 per cent of others.

‡ All cocci gram-positive and 33.33 per cent of others.

§ All cocci gram-positive and 59.25 per cent of others.

‖ One questioned.

** One culture from 24 inches produced gas in glucose and sucrose.

rods, 1 spore former, and 1 coccus; 12 inches, 3 short rods and 1 spore former; 24 inches, 1 coccus, 1 pleomorphic form, 1 short rod, and 7 spore formers.

On account of the great diversity of reactions it was not possible to arrange the cultures in large groups. The groups that might be formed by those most alike culturally showed great diversity in morphology.

COMPARISON OF THE ATLANTIC AND PACIFIC SOILS

The Pacific sand, a fine-grained, acid soil containing very little soluble salts and almost no combustible material, agrees in general more closely with that from the northern Atlantic than that from the southern. For example, the water relations of the two are very similar. The total count for the Pacific soil lies between those for the two Atlantic soils, but the high percentage of actinomycetes is noticeable. This again (1, IV) can be attributed neither to water content nor to high pH value, although the percentage was slightly higher in October, after the summer's drying. The plates of the Pacific soil showed a much lower percentage of colored forms than did those of either of the Atlantic soils, but the colors of the cultures studied agreed more nearly with those from the northern than the southern Atlantic area.

Morphologically the cultures from the three soils vary rather widely although the percentages of spore formers in the Pacific and northern Atlantic areas agree fairly closely. The much greater fermentative activity of the Pacific soil is a marked difference between the soils compared, particularly in the case of the 25 per cent lactose fermenters and the one culture that formed gas in glucose and sucrose. With the exception of the rather unusual form noted in the North Carolina sand (1, IV) this is the only case of gas formation encountered in the entire study. There is in the Pacific sand noticeably less digestive action but approximately the same amount of reduction as in the northern soil. The southern Atlantic sand differs rather widely on these two points from the other two soils.

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THE AMPHOTERIC NATURE OF THREE COASTAL PLAIN SOILS: I. IN RELATION TO PLANT GROWTH

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In a former publication (4, XII) the relation of the amphoteric nature of certain soil colloids (Sharkey, Sassafras, and Nipe) and the ionization of the basoid group, together with the resultant injury to plant growth was indicated. The soils used in that study were mineral soils from widely separated places representing soils in different categories and characterized by colloids with different silica sesquioxide ratios (3.18, 1.89, 0.31). From the standpoint of aluminum toxicity, mineral soils with colloids of low silica/alumina ratios show toxicity at higher pH values than do soils with wide ratios. In a study of some of the factors that influence the growth of beets, spinach, and other vegetable crops under field conditions within the coastal plain of Virginia, certain relations between soil type, organic matter, pH values, and plant growth were discovered. These crops were found to grow more satisfactorily on some soil types than on others, and in all cases the soils with high organic matter supported crops at lower pH values. The purpose of the experiment herein reported is to note the interrelation between the amphoteric properties of different soil types found locally and the growth of vegetable crops.

The particular soils used in this study were virgin soils[1] and were classified as follows: Portsmouth loamy fine sand (2), Bladen sandy loam (5), and Norfolk very fine sand (2). Briefly, these soils may lie in rather close proximity but by virtue of their position have developed rather definite characteristics. As a result of good surface and internal drainage the Norfolk soils have developed a mature profile comparatively highly weathered. Because of their low organic matter content and comparatively high silica-sesquioxide ratio, they have a relatively high ultimate pH value (4, XII). The Portsmouth soils may have the same parent material in many instances as the Norfolk soils, but by virtue of their low position have not developed a mature profile. They have, however, accumulated a high organic matter content. These two factors give rise to a colloid of a distinctly different nature from the Norfolk—of a more acid character and lower ultimate pH. The Bladen soils generally are derived from beds of heavy clay or heavy fine clay which lie close to tidal

[1] The author is grateful to Messrs. H. E. Hearn and H. G. Byers of the Bureau of Chemistry and Soils, U. S. Department of Agriculture for information concerning the development of these soils.

marsh. These materials have undergone very little aeration and oxidation and in some cases only a slight amount of eluviation in the surface soil which has given rise to a colloid of a rather definite composition. The soil herein used had a relatively low ultimate pH value, was rather low in organic matter, and possessed a colloid of comparatively wide silica/alumina ratio. Table 1 gives comparative data for the three soils.

EXPERIMENTAL PROCEDURE

The soils were brought to the greenhouse and while still moist were placed in 2-gallon earthenware crocks. Large rocks, then fine rocks, and finally silica sand were placed in the bottom of the crocks to insure uniform leaching. The soils were treated with lime or organic matter, as indicated in table 2, before being placed in the crocks. The soil reactions were varied by the use of hydrated lime. The moisture was maintained at an optimum content through-

TABLE 1

Data concerning soils under study

(Results Calculated to Soil Dried at 110°C.)

NAME OF SOIL SERIES	SAND*	SILT	CLAY	LOSS ON IGNITION	ORGANIC MATTER	NITROGEN	SOIL pH		REPLACEABLE BASES M.E. 1,000 GM. SOIL			
							Virgin State	Ultimate† State	CaO	MgO	K_2O	Exchange capacity (to pH 7.0)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*						
Portsmouth..	83	4	5	13.60	7.71	0.319	4.1	3.5	15.3	9.2	2.9	274
Bladen.....	75	11	12	3.10	1.80	0.059	4.4	3.6	6.8	6.6	2.0	102
Norfolk.....	90	4	5	1.60	0.93	0.048	5.0	4.7	5.1	5.9	1.4	48

* By the pipette method. Organic matter destroyed by H_2O_2.

† Electrodialyzed soil and water ratio 1:2. pH by quinhydrone and colorimetric methods.

out the growth period. The following data briefly summarized the treatment of the soils:

Beet Crop.—Five beet plants 2 cm. high were set June 13, 1933; fertilized June 26 and July 7; harvested July 25. Each pot received 1.02 gm. of nitrogen, 0.93 gm. P_2O_5, and 0.93 gm. K_2O. The P_2O_5 was from ammophos; K_2O from KCl; N from ammophos, Na NO_3, and $(NH_4)_2$ SO_4. The weight of soil (110°C.) in the pots was as follows: Portsmouth 5,200 gm., Bladen 6,800, and Norfolk 7,700. On 7/17/33 the soils were leached with rain water until a volume of about 1,500 ml. was obtained, or an equivalent of nearly 2 inches of rain. Certain of the results are given in table 2.

Strawberry Crop.—After the removal of the beets, three well-developed strawberry plants (Heflin) were set in each pot on July 27 and harvested on October 30. The crop neither received any further fertilizer treatment nor was the soil leached during or after its growth. The yield data are given in table 2.

TABLE 2

Effect of Soil Acidity and Organic Matter on the Growth of Crops and the Solubility of Aluminum in Different Soil Types

BEETS			STRAWBERRIES		SPINACH		
Dry total weight—roots and tops	Mean pH	Al_2O_3 leached	Dry weight—tops	Mean pH	Dry weight—tops	Mean pH	Al_2O_3 leached
			Portsmouth				
gm.		*mgm.*	*gm.*		*gm.*		*mgm.*
0.1	4.0	11.62	1.3	4.0	0.1	4.0	148.96
6.9	4.8	0.22	6.2	4.6	0.6	4.4	1.58
11.6	5.4	None	9.1	5.2	4.5	5.0	0.06
12.0	5.8	None	8.4	5.6	5.2	5.5	None
12.7	6.1	None	10.8	5.7	5.3	5.7	None
13.3	6.2	None	9.6	5.9	6.1	5.8	None
			Bladen				
....	4.2	0.86	1.6	4.4	...	4.4	65.35
4.8	4.9	0.06	2.3	4.8	...	4.5	29.00
7.0	5.1	None	4.6	4.9	0.6	4.8	15.70
8.4	6.1	None	6.7	5.6	3.5	5.4	0.03
7.2	6.7	None	3.5	6.3	4.3	6.3	None
			Organic matter at low pH (1, 2, 4 per cent)				
1.4		None	4.2				
8.9		None	5.1				
10.8		None	7.1				
			Organic matter at optimum pH (1, 2, 4 per cent)				
9.2		None	6.3		3.3		None
10.5		None	6.6		3.5		None
12.7		None	8.3		3.7		None
			Norfolk				
0.2	5.5	2.37	....	5.2	...	4.8	302.68
3.5	5.7	None	1.1	5.4	...	5.0	3.33
4.5	6.2	None	2.4	5.6	3.9	5.5	0.26
3.7	6.6	None	7.2	5.8	6.7	6.0	None
3.0	7.0	None	6.7	6.2	5.6	6.2	None
			Organic matter at low pH (1, 2, 4 per cent)				
6.6		None	2.3		0.8		11.52
9.1		None	6.0		1.6		8.06
11.9		None	11.9		2.4		1.33
			Organic matter at optimum pH (1, 2, 4 per cent)				
9.5		None	6.0		5.0		None
12.7		None	10.5		6.7		None
12.8		None	11.6		5.8		None

Spinach Crop.—On November 13 the soil in each pot was planted to 10 hills of spinach (Virginia Savoy). The seeds were treated with red oxide of copper to prevent damping off. On November 25 each pot was fertilized with one-half the quantity of fertilizer from the same source as that used on the beets. The pots were leached a few days before harvest until about 1,500 ml. of water was collected. The yield data and aluminum leached are also shown in table 2.

Soil reaction and plant growth

The soil colloid constitutes complexes of weak acids (silicic, humic, etc.) and bases (aluminum, iron, etc.) which only partially neutralize each other, leaving free valences for the absorption of bases at high pH values (4, X). The extent of the excess of acids over the bases and the quantity of the material in the soil control the absorbing power of a soil for lime. The Portsmouth soil has a base holding capacity at pH 7, nearly three times that of the Bladen, principally because of the higher organic content (1, 8). The ultimate reaction (4, X) of the soils was pH 3.5 for the Portsmouth and pH 3.6 for the Bladen, whereas that for the Norfolk soil was pH 4.7. The differences in the composition of these soil colloids were reflected in the point of exchange neutrality (the pH of the electrodialyzed soil in a *N* Na_2SO_4 solution), which was Portsmouth 3.8, Bladen 4.2, and Norfolk 5.0. The lowest pH value that these soils can show without the disintegration of the soil colloid is reflected by this point. This in turn indirectly effects the lowest pH at which plants will make good growth. These readings are about as indicative of the character of the soil colloids as is a chemical analysis. They reflect that the acidoid/basoid ratio widens progressively as follows: Norfolk, Bladen, Portsmouth. The organic or humus content of the Portsmouth is principally responsible for its high acidoid content, which would not necessarily be reflected in the silica-sesquioxide ratio.

For the beet crop the lowest reaction for good growth in the Portsmouth was pH 4.8, or about 25 per cent saturated in respect to calcium; pH 4.9, or 20 per cent saturated, for the Bladen; pH 5.7, or 18 per cent saturated, for the Norfolk soil. Very marked increases in yields were obtained at higher reactions, however. A reaction of pH 6.0 or slightly above (9) seems to be very favorable for the growth of beets in these soils.

The addition of organic matter to the Bladen and Norfolk soil lowered the reaction at which beets grew satisfactorily. The source of organic matter was peat moss which was exceedingly low in available plant nutrients and had a low pH value. The peat moss carried a pH of 3.8, 0.707 per cent nitrogen, and 5.43 per cent ash content. The benefit of the peat moss, then, was not in the nutrients that it carried but in the physico-chemical action on the soil colloid; that is, it increased the active (1, 3, 8) acidoid constituent. The fact that it did not greatly increase the yields at the optimum reaction in the Bladen, but did in the Norfolk soil, further supports this view.

For the strawberry crop the lowest reactions for good growth were pH 4.6, 4.9, and 5.8 for the Portsmouth, Bladen, and Norfolk soils respectively. Although the strawberry plant is often considered to be very tolerant of an acid soil, these results indicate that it is about as sensitive to an unfavorable soil condition as are beets. Probably the conception that the strawberry plant will grow at low pH values has arisen from the fact that strawberries are usually grown on low-lying soils that carry a high organic content. The response to organic matter in the Bladen and Norfolk soils further supports this view.

The grower has found that spinach, possibly more than most of the vegetable crops, has responded to liming practices. The results shown in table 2 indicate at least that this crop is very responsive to a change in soil reaction toward the neutral pH. The lowest pH readings for good growth were 5.0, 5.4, and between 5.5 and 6.0 for the Portsmouth, Bladen, and Norfolk soils. Spinach showed also the characteristic response to increased organic matter content of the soils.

Soluble aluminum

The significance of the effect of soluble aluminum in acid soils upon plant growth has been pointed out by many workers (4, XII; 6). Attention has also been called to the fact that aluminum and other components of the basoid group ionize and become soluble at different pH values in soil types with different isoelectric points. For example, a soil with a high ultimate pH represents one with a relatively active basoid group in which the aluminum is ionizable and soluble at a comparative high pH value and *vice versa* for a soil with a low ultimate pH value. Further indications of these facts are noted in the pH of exchange neutrality. As shown in table 2, aluminum appeared in solution at pH 4.8 in the Portsmouth soil, at pH 4.9 in the Bladen, and in comparatively large quantities at pH 5.5 in the Norfolk soil during the growth of the beet crop and at a slightly higher pH for the spinach crop. At a pH of 5.5 neither the Bladen nor the Portsmouth soil showed any aluminum in the drainage water during the growth of beets. Further, plant growth was very good at this reaction in these two soils, which is all in agreement with the amphoteric nature of the soil. A somewhat similar relation was observed between these points for the Sharkey, Sassafras, and Nipe soils (4, XII).

The addition of a strongly acidoid material to a soil of the Norfolk type increases the acidic properties, thereby suppressing the activity of the basoid group. This action suppresses the ionization and solubility of the basoid group, which in turn suppresses the fixing power of the soil for phosphorus. Data on the leaching of phosphorus from the soil is given in part II of this paper.

The data in table 2 show that much larger quantities of aluminum were leached from the soil at low pH values after the growing of the spinach than after the beet crop. Several factors account for this large difference. The lowering of the soil reaction from the effects of acid-forming fertilizers and of

leaching certainly influenced the leaching of aluminum. A small lowering of the soil reaction at the critical point makes a big difference in the leaching of the basoid constituent. Further, less phosphorus was added during the growth of the spinach than in the growing of the beets. Phosphorus was practically negative in the leaching water from the spinach, whereas it was much higher in the drainage water from the beets. Many experimental data (6, 9) show that through the addition of heavy applications of phosphorus to acid soils plants grow better and that this growth is due to the removal of aluminum from solution. Since the Norfolk soil has a strong power for fixing phosphorus (*see* part II), the growth of the crops at the optimum pH was not so good as on the other two soils. When organic matter was added to the Norfolk soil, thereby lessening the power to fix phosphorus, the crops grew as well as on the other soils. Further work in progress shows clearly that low availability of phosphorus was responsible for the depressed yields in the Norfolk soil. Part II deals with the leaching and absorption of soil constituents on the different soil types.

SUMMARY

Three soils found in the coastal plain of Virginia, a Portsmouth loamy fine sand, a Bladen sandy loam, and a Norfolk very fine sand, with ultimate pH values of 3.5, 3.6, and 4.7, respectively, were selected for a study of the effect of soil acidity upon the growth of vegetables. The lowest reactions for satisfactory growth of beets on the Portsmouth series was pH 4.8; on the Bladen series, 4.9; and on the Norfolk, 5.7, or at approximately the same percentage of calcium saturation. For strawberries the lowest reaction for good growth was pH 4.6, 4.9, and 5.8; for spinach 5.0, 5.4, and between 5.5 and 6.0, respectively, for the Portsmouth, Bladen, and Norfolk soils. The point at which growth was markedly retarded was directly correlated with the appearance of aluminum in the drainage water. The addition of organic matter, in the form of peat moss, to the Bladen and Norfolk soils suppressed aluminum solubility at low pH values and enabled crops to grow more satisfactorily than in the untreated soils.

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PLATE 1

EFFECT OF SOIL TYPE, REACTION, AND ORGANIC MATTER UPON THE GROWTH OF BEETS

FIG. 1. Effect of soil type and reaction.

FIG. 2. Effect of soil type and organic matter (peat moss source of organic matter).

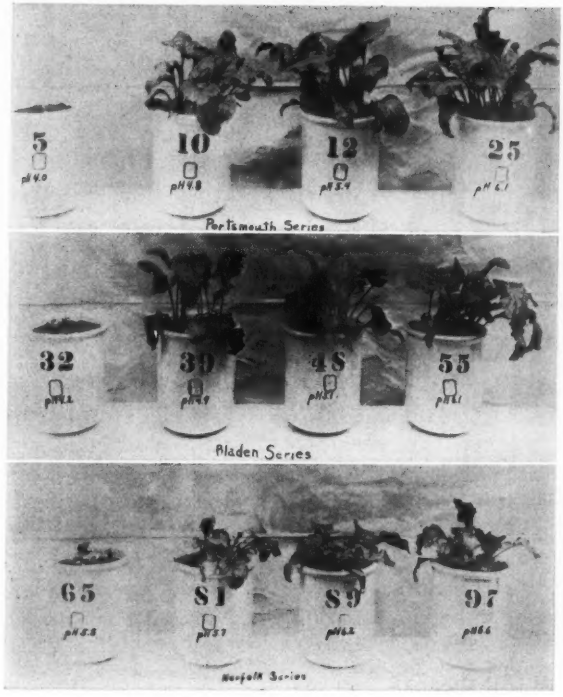

FIG. 1

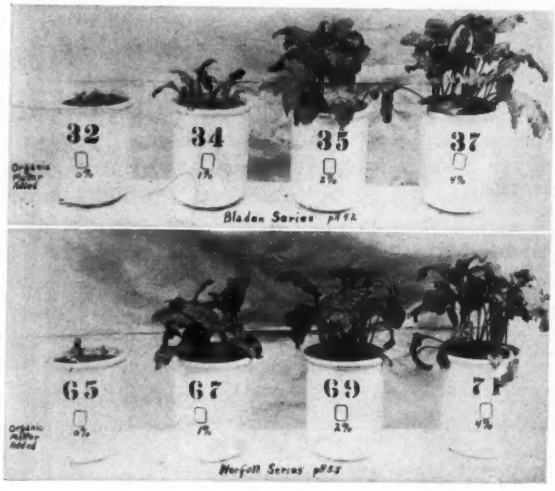

FIG. 2

UNIV. OF MICH.

THE AMPHOTERIC NATURE OF THREE COASTAL PLAIN SOILS: II. IN RELATION TO THE LEACHING AND ABSORPTION OF SOIL CONSTITUENTS BY PLANTS

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ANALYTICAL DATA

As explained in Part I, a crop of beets was grown upon the soil until it was evident that the maximum growth had been reached. The soil was then leached with approximately 1,500 ml. of rain water, the equivalent of almost 2 inches of rain. The chemical analyses[1] of the drainage water and plant material for certain nutrients are given in table 3. The first row of figures in each soil series mentioned represents the virgin soil with no treatment other than fertilization. Table 4 gives the percentage of the replaceable bases removed by plant absorption and leaching. The assumption that the potash applied was replaceable is possibly incorrect. The Bladen soil apparently has a high fixing power for potash. Table 5 gives the percentage of total nitrogen and the percentage of the added phosphorus leached and absorbed by the plant. The soils were leached while the spinach was growing and the analytical results are given in table 6. The data in tables 3–6 are given to show the behavior of the three soil types under these conditions and are discussed from that point of view.

Calcium

The quantity of calcium leached increased with a rise in pH but not in proportion to the quantity of lime added. For example, as expressed in table 4, 15 per cent of the replaceable lime in the Portsmouth soil was leached at pH 4.0, whereas only 2 per cent was leached at pH 6.2. A similar relation held true for the Bladen and Norfolk soils. There was an increase in absorption by the plants with a rise in pH until the optimum growth was attained, or in other words, the higher the crop yield the greater the absorption of calcium. The total quantity of replaceable calcium was in the order Portsmouth, Bladen, Norfolk. The leaching of the lime maintained the same order, but the percentage of the total replaceable leached was in the reverse order. In other words, the soil that had the greatest exchange capacity had the strongest combining power for lime. Since the Norfolk soil has a low base exchange capacity

[1] Florence A. Shelton assisted in making the chemical analyses.

the removal of calcium (on the percentage of total replaceable) was the highest of the three soils at any given pH value.

Magnesium

The results of the leaching and absorption of magnesium indicate that plant absorption is a big factor in the removal of magnesium from the soil. Since no magnesium was added, all removed had to be accounted for by that present

TABLE 3

Effect of soil type and soil reaction upon the leaching and absorption of nutrients by plants

(After beet crop)

MEAN pH	M.E. PER 1,000 GM. OF SOIL (110° C.)									
	Calcium		Magnesium		Potassium		Phosphorus		Nitrogen	
	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed
					Portsmouth					
4.0	2.38	....	1.09	....	1.73	....	0.01	....	6.78	....
4.8	3.88	0.91	0.60	0.46	0.66	1.07	0.11	0.34	5.20	4.19
5.4	3.41	1.34	0.45	1.09	0.69	2.30	0.29	0.46	3.71	6.24
5.8	3.72	1.43	0.35	1.00	0.41	2.75	0.23	0.47	3.69	6.75
6.1	4.33	1.57	0.34	1.22	0.34	3.35	0.18	0.47	4.41	6.53
6.2	6.15	1.71	0.40	1.20	0.34	3.68	0.13	0.53	6.31	7.30
					Bladen					
4.2	1.31	....	0.56	....	0.38	....	0.03	....	2.48	....
4.9	1.12	0.27	0.29	0.39	0.21	0.43	0.03	0.14	1.12	2.13
5.1	1.20	0.49	0.21	0.51	0.26	0.70	0.03	0.21	1.64	3.27
6.1	2.28	0.64	0.26	0.49	0.12	1.00	0.06	0.25	1.04	3.80
6.7	2.41	0.63	0.19	0.32	0.13	0.95	0.07	0.21	1.89	3.45
					Norfolk					
5.5	1.13	....	0.53	....	1.06	....	0.005	0.01	4.31	....
5.7	1.92	0.23	0.37	0.15	0.81	0.33	0.01	0.09	1.91	1.54
6.2	2.49	0.27	0.34	0.19	0.52	0.75	0.01	0.11	2.92	1.91
6.6	4.37	0.19	0.32	0.21	1.10	0.58	0.015	0.09	4.49	1.55
7.0	3.99	0.15	0.31	0.17	0.88	0.47	0.025	0.07	4.65	1.21

in the soil. The leaching of magnesium at low pH values was very high in proportion to the total removal at high pH values. The fact that a great deal of the available magnesium is removed from the soil by leaching has been shown in the Norfolk area in connection with magnesium deficiency on potatoes. During rainy years this deficiency becomes apparent over the entire trucking area, but is observed to a much more limited extent in dry years (2). The analyses of the drainage water and plant material were, therefore, a good indication of the relative available magnesium under these conditions.

Potassium

The Bladen soil possesses a high power for fixing potash in a non-replaceable state. Many soils apparently (8, 11, 12, 14, 16) show this property. The total removed from this soil is low in comparison to the other two soils. The removal from the Portsmouth by leaching is fairly low, but plant absorption is high, the total utilization by one crop of beets being 85.2 per cent of the replaceable and added potassium at the optimum reaction. The total removed

TABLE 4

Effect of soil type and soil reaction upon the leaching and absorption of replaceable bases

(After beet crop)

MEAN pH	PER CENT OF REPLACEABLE BASES* IN SOIL REMOVED BY LEACHING AND PLANT ABSORPTION								
	Calcium			Magnesium			Potassium		
	Leached	Absorbed	Total	Leached	Absorbed	Total	Leached	Absorbed	Total
				Portsmouth					
4.0	15.55	0.00	15.55	11.85	0.00	11.85	36.7	0.0	36.7
4.7	4.44	1.04	5.48	6.53	5.00	11.53	14.0	22.7	36.7
5.4	2.11	0.82	2.93	4.90	11.85	16.75	14.6	48.8	63.4
5.8	1.79	0.69	2.48	3.81	10.76	14.57	8.7	58.3	67.0
6.1	1.69	0.61	2.30	3.70	13.27	16.97	7.2	71.0	78.2
6.2	2.03	0.56	2.69	4.35	13.05	17.40	7.2	78.0	85.2
				Bladen					
4.2	18.67	0.00	18.67	8.49	0.00	8.49	11.2	0.0	11.2
4.9	3.86	0.97	4.83	4.40	5.91	10.31	6.2	12.7	18.9
5.1	2.34	0.96	3.30	3.18	7.73	10.91	7.7	20.7	28.4
6.1	3.11	0.87	3.98	3.94	7.43	11.37	3.5	29.5	33.0
6.7	1.72	0.45	2.17	2.88	4.70	7.58	3.8	28.0	41.8
				Norfolk					
5.5	22.05	0.00	22.05	9.00	0.00	9.00	42.6	0.0	42.6
5.7	12.99	1.55	14.53	6.10	2.54	8.64	31.2	13.3	44.5
6.2	10.21	1.10	11.31	5.76	3.22	8.98	19.8	28.5	48.3
6.6	9.95	0.46	10.41	5.42	3.56	8.89	41.5	22.0	63.5
7.0	4.84	0.19	5.03	5.26	2.88	8.14	33.1	17.9	51.0

* Assuming that added nutrients were in replaceable state.

in the Norfolk soil was high, with leaching accounting for the greater proportion removed. This method of studying the relative available potash for the crops used was exceptionally well adapted to these soils.

Phosphorus

The Portsmouth, Bladen, and Norfolk soils received an application of 7.7, 5.9, and 5.2 m.e. of phosphorus per 1,000 gm. of soil for the beet crop. The

data in table 3 show that only a small proportion of this was removed either through leaching or by the plants. The absorption by the plants and the leaching were influenced markedly by the soil reaction, organic matter content, and amphoteric point of the soil. The amount of phosphorus removed from the soil by leaching following the growth of spinach was so small that it is not mentioned except in the Portsmouth soil.

A great deal has been said (3, 5, 6, 14, 17) about the fixation of phosphates in different soils. Sundry compounds (4, 6, 12, 13) of different natures have

TABLE 5

Effect of soil type and soil reaction upon the leaching and absorption of nitrogen and phosphorus

(After beet crop)

PER CENT TOTAL NITROGEN IN SOIL REMOVED			PER CENT OF ADDED PHOSPHORUS REMOVED		
By leaching	By plant	Total	By leaching	By plant	Total
		Portsmouth			
2.72	0.00	2.72	0.08	0.00	0.08
2.10	1.68	3.78	0.87	2.70	3.57
1.49	2.51	4.00	2.30	3.65	5.95
1.48	2.71	4.19	1.83	3.73	5.56
1.76	2.62	4.38	1.43	3.73	5.16
2.53	2.93	5.46	1.03	4.21	5.24
		Bladen			
4.63	0.00	4.63	0.31	0.00	0.31
2.10	3.98	6.08	0.31	1.46	1.77
3.05	6.10	9.15	0.31	2.20	2.51
1.93	7.07	9.00	0.62	2.60	3.22
3.52	6.40	9.92	0.73	2.20	2.93
		Norfolk			
9.92	0.00	9.92	0.02	0.12	0.14
4.42	3.55	7.97	0.12	0.95	1.07
6.68	10.05	16.73	0.12	1.17	1.29
10.30	13.85	24.15	0.18	0.95	1.13
10.65	13.54	24.19	0.26	0.82	1.08

been shown to account for this absorption. Since clays show amphoteric properties (1; 7; 11, VI) the question of the fixation of phosphates in acid soils is indicated. For example, soils with a high amphoteric point (laterites, etc.) fix larger amounts of phosphates than do soils with low amphoteric points (7; 11, VI). Because of the amphoteric nature of soils, more phosphorus is fixed at low pH values than at high reactions. Soils with low organic content fix greater quantities of phosphorus than do similar soils with high organic content. The addition of electronegative material, silicates and humates, reduced the quantity of anion fixed by the colloid.

We find only a low utilization of phosphorus in all of these soils. However, the results in table 4 and the calculations in table 5 indicate that the soils have fixing powers for phosphorus which may be expressed in the order Norfolk, Bladen, Portsmouth. The utilization by the plants and the leaching of phosphorus were in the reverse order of fixation. The additions of a strongly electronegative material (humus) which satisfied some of the positive valences of the colloidal complex prevented somewhat the fixation of the phosphorus, thus permitting its better utilization by the plants. The low availability of

TABLE 6

Effect of soil type and soil reaction upon the leaching and absorption of nutrients by plants

(After spinach crop)

MEAN pH	M.E. PER 1,000 GM. OF SOIL (110°C.)									
	Calcium		Magnesium		Potassium		Phosphorus		Nitrogen	
	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed
					Portsmouth					
4.0	2.18	....	1.24	....	0.97	....	0.002	....	6.75	....
4.4	7.36	....	1.30	....	0.68	....	0.006	....	7.45	....
5.0	7.07	0.86	0.80	0.54	0.21	1.42	0.008	0.31	4.84	2.88
5.5	6.54	1.18	0.57	0.68	0.14	1.73	0.05	0.39	4.10	3.60
5.7	6.65	1.22	0.51	0.69	0.09	1.80	0.061	0.40	4.40	3.10
5.8	7.90	1.41	0.71	0.85	0.10	2.11	0.062	0.41	4.74	3.98
					Bladen					
4.4	2.35	....	0.94	....	0.71	....	.....	....	4.88	....
4.5	3.97	....	0.78	....	0.41	....	.....	....	4.67	....
4.8	4.13	0.09	0.56	0.03	0.14	0.06	.....	0.03	3.84	0.19
5.4	4.17	0.46	0.32	0.32	0.06	0.62	.....	0.16	2.91	1.46
6.3	5.70	0.67	0.48	0.32	0.09	0.95	.....	0.27	3.06	1.63
					Norfolk					
4.8	1.65	....	0.64	....	1.23	....	.....	....	4.12	....
5.0	4.15	....	0.86	....	0.81	....	.....	....	5.35	....
5.5	4.97	0.26	0.80	0.22	0.41	0.50	.....	0.11	4.77	1.06
6.0	5.28	0.50	0.62	0.46	0.22	1.55	.....	0.28	3.54	2.66
6.2	6.77	0.45	0.69	0.34	0.15	1.28	.....	0.21	2.99	2.16

phosphorus in the Norfolk soil was no doubt responsible for low crop yield. Then the addition of organic matter overcame this deficiency by preventing to some extent the fixation of the phosphorus, and thereby brought up the crop yield to the maximum for any of the three soils. A very high fixation of phosphates at low pH values was noted in all of these soils. The estimation of the phosphorus by chemical analyses of the leaching water and plant material was very satisfactory under these conditions for determining its relative availability.

Nitrogen

The carbon/nitrogen ratio of the three soils used—Portsmouth, Bladen, and Norfolk—was 14, 17, and 11 respectively. From this standpoint (15, 16) the availability of nitrogen would be the least in the Bladen and the highest in the Portsmouth and Norfolk soils. The largest amount of removed nitrogen, found in the Portsmouth soil, was due to the high state of fertility of the soil. The addition of lime greatly stimulated the bacterial activity (16) and likewise the nitrogen released from the organic matter of the soil. Since there were added 14, 11, and 10 m.e. of nitrogen per 1,000 gm. of soil for the Portsmouth, Bladen, and Norfolk, the removal was almost 100 per cent for the Portsmouth, about 50 per cent for the Bladen, and more than 60 per cent for the Norfolk soil, at relatively high pH values during the growth of the beet crop. The addition of organic matter stimulated high utilization of nitrogen on the Norfolk and Bladen soils, not because of the extra added nitrogen, but because of its physico-chemical action on the soil.

DISCUSSION

The colloidal complexes represented in these three soils differ both in quality and quantity as indicated by their amphoteric points and exchange capacity. These differences manifest themselves in various ways: First, in the quantity of lime necessary to neutralize the acidity of the soil. The CaO holding capacity of the soils in pounds per acre, 0–$6\frac{2}{3}$ inch basis, at pH 7.0 on the basis of their specific gravity was 11,850, 5,720, and 2,760 for the Portsmouth, Bladen, and Norfolk soils respectively. This difference is accounted for quantitatively in the amount of colloid in the soil and qualitatively by the difference in the composition of the colloid. Although the Portsmouth soil carried more replaceably lime, the leaching of the lime from the soil was, in terms of the percentage of the total replaceable, less than that from the Bladen, which was in turn less than that of the Norfolk soil. This is accounted for by the fact that the Portsmouth soil carried a colloid with relatively strong acidic properties, low ultimate pH, and low isoelectric point. Secondly, the Bladen soil had a low ultimate pH but less active colloid than did the Portsmouth soil. The Norfolk soil had a relatively high ultimate pH and a smaller quantity of colloid to a given amount of soil. These two factors, then, account for its low exchange capacity. Although all of the soils have a relatively strong power for fixing phosphorus, the Norfolk soil showed the greatest power. At low pH values all three soils showed very strong phosphate fixing powers.

Since the soils show differences in their power to give up cations (9; 11, XI) and in their power to fix phosphates (7, 11, 16, 15, 20), and further, since crops show different nutrient requirements (15) and different powers to absorb (15, 18) them, and since these differences are shown by the analyses of the leaching water and plant material, the author believes that some form of the aforementioned method will be valuable in determining the relative availability of plant nutrients and toxants for a specific crop.

Leaching of Organic Matter

As a result of the favorable reaction in the soil brought about by the applications of lime, the bacterial activity of the soil was greatly increased (21) as the reaction approached neutrality. This affected the soil processes in several ways, such as the liberation of plant nutrients and other soil constituents. As the soil reaction in each soil type approached the neutral pH reading, the amount of organic matter in the leaching water definitely increased. These results were somewhat more pronounced in the first leaching than in the second. These data are given in table 7. The leaching of the organic matter is associated with the amphoteric points of the soils. For example, there is about as much organic matter leached at pH 6.2 in the Norfolk soil as at pH 6.1 in the Portsmouth soil even though the Portsmouth soil contains about seven times as much organic matter. Again, at the reaction pH 5.5 in the Norfolk soil comparatively little organic matter is leached, whereas a reaction of pH 4.0 was reached before the Portsmouth soil showed as low leaching of organic matter. The Bladen soil showed little or no organic matter leached at pH 4.2.

TABLE 7

Influence of soil reaction upon the leaching of organic matter

PORTSMOUTH		BLADEN		NORFOLK	
Mean pH	Organic matter leached	Mean pH	Organic matter leached	Mean pH	Organic matter leached
	mgm.		*mgm.*		*mgm.*
4.0	5.85	4.2	0.00	5.5	6.66
4.8	13.14	4.9	2.02	5.7	22.90
5.4	29.82	5.1	4.04	6.2	27.30
5.8	23.61	6.1	6.00	6.6	81.10
6.2	41.80	6.7	10.20	7.0	112.00

SUMMARY

Three soils found in the coastal plains of Virginia represent soils with wide variations in cation exchange capacity, replaceable bases, pH value, and organic matter content. These soils show different amphoteric points, which reflect differences in the leaching and absorption of soil constituents.

Data are given to show the advantage of using the analysis of the plants and the drainage water from pot cultures to show the limiting element in plant growth and the available nutrients and toxants for specific crops in these soils.

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